# Genome-Wide Survey and Expression Analysis of Amino Acid Transporter Gene Family in Rice (*Oryza sativa* L.)

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## Abstract

*Background:* Amino acid transporters (AATs) that transport amino acids across cellular membranes are essential for plant growth and development. To date, a genome-wide overview of the *AAT* gene family in rice is not yet available.

*Methodology/Principal Findings:* In this study, a total of 85 *AAT* genes were identified in rice genome and were classified into eleven distinct subfamilies based upon their sequence composition and phylogenetic relationship. A large number of *OsAAT* genes were expanded via gene duplication, 23 and 24 *OsAAT* genes were tandemly and segmentally duplicated, respectively. Comprehensive analyses were performed to investigate the expression profiles of *OsAAT* genes in various stages of vegetative and reproductive development by using data from EST, Microarrays, MPSS and Real-time PCR. Many *OsAAT* genes exhibited abundant and tissue-specific expression patterns. Moreover, 21 *OsAAT* genes were found to be differentially expressed under the treatments of abiotic stresses. Comparative analysis indicates that 26 *AAT* genes with close evolutionary relationships between rice and *Arabidopsis* exhibited similar expression patterns.

*Conclusions/Significance:* This study will facilitate further studies on *OsAAT* family and provide useful clues for functional validation of *OsAATs*.

## Introduction

Amino acid transporters (AATs) are the integral membrane proteins which mediate the transport of amino acids across cellular membranes in higher plants, and play an indispensable role in various processes of plant growth and development, including long distance amino acid transport, response to pathogen and abiotic stresses [1–3]. By heterologous expression systems and database screening with known transporters, more than 60 distinct AAT genes have been identified in *Arabidopsis* [4], as well as several genes in other plant species.

Plant AATs family includes two main families that belong to the amino acid-polyamine- choline (APC) transporter superfamily: the amino acid/auxin permease (AAAP) family and the APC family [5,6]. There are at least six subfamilies in the AAAP family, including amino acid permeases (AAPs), lysine and histidine transporters (LHTs), proline transporters (ProTs), γ-aminobutyric acid transporters (GATs), auxin transporters (AUXs), and aromatic and neutral amino acid transporters (ANTs). The APC family in plants is grouped into three subfamilies: cationic amino acid transporters (CATs), amino acid/choline transporters (ACTs) and polyamine H⁺-symporters (PHSs) [7–9].

Previously, molecular functions of many AATs were fully characterized in *Arabidopsis*. The eight members (*AtAAP1-AtAAP8*) were found in the *AtAAP* subfamily, and six AtAAPs were demonstrated to transport neutral and charged amino acids with varying specificities and affinities [10–12]. *AtAAP1* was highly expressed in the cotyledons and the endosperm and regulated the import of amino acid into root cells or developing embryo [13–15]. *AtAAP5* might transport amino acids into the developing embryo, and be the most important component in the root amino acid uptake system [16]. The analysis of *aap6* mutant demonstrated the physiological role of AtAAP6 in regulating sieve element composition [9]. *AtAAP8* might be required for amino acid uptake into the endosperm and developing embryos at early stages [17]. In addition, the functions of AAP gene members from other plant species had also been studied, such as *StAAP1* [18], *VfAAP1* and *VfAAP3* [19], *PvAAP1* [20]. Recently, it was proposed that *PtAAP11* played a major role in xylogenesis by providing proline in poplar [12].

AtLHT1, a specific transporter for lysine and histidine, was a key mediator of root uptake of amino acids and supply of leaf mesophyll with xylem-derived amino acids in *Arabidopsis* [21,22]. AtLHT1 and AtAAP5 together played critical and separate roles in root uptake of cationic amino acids at concentrations typically possessed in soils [23,24]. AtProTs were responsible for transporting proline, glycine betaine and GABA, and their expression patterns were complementary [25]. Although intracellular localization and substrate specificity were very similar, *AtProTs* fulfilled different roles in planta [26,27]. It was reported that the rice ProT

protein (OsProT) has 68.8% and 59.6% similarity to AtProT1 and LeProT1, respectively, and specifically transported L-Pro in a transport assay by using Xenopus laevis oocytes [28,29]. In addition, heterologous expression analysis of HvProT indicated that it might play a crucial role in the transport of proline to root tip region and involve in organ development [30]. AtGAT1 encoded high affinity γ-aminobutyric acid (GABA) transporter and was highly expressed in flowers and under the condition of high GABA concentrations such as wounding and senescence [31]. AtANT1 was specifically expressed in flowers and cauline leaves, and transported aromatic and neutral amino acids as well as arginine and indole-3-acetic acid [32]. AtAUX1 was known as an auxin influx carrier and mediated the import of IAA which was a tryptophan-like signaling molecular [33]. AtAUX1 regulated root gravitropism and promoted lateral root formation by facilitating IAA distribution between sinks and source tissues in the Arabidopsis seedling [34,35]. AtLAX3, a homologue of AtAUX1, was likely to influence the rate of lateral root emergence by regulating the auxin inducible expression of cell-wall-remodeling genes [36,37]. In the wild cherry Prunus avium, PaLAX1 promoted the uptake of auxin into cells and affected the content and distribution of free endogenous auxin [38]. In the AtCAT subfamily, nine members were identified based on their sequence similarity. AtCAT5 functioned as a high-affinity, basic amino acid transporter in an amino acid transport in yeast [39]. AtCAT3, AtCAT6 and AtCAT8 preferentially transported neutral or acidic amino acids [40]. Meanwhile, AtBAT1, similar to a yeast GABA transporter (UGA4), was isolated as a bi-directional amino acid transporter [41]. Recently, this gene was characterized to be expressed in the mitochondrial membrane and mediate the transport of GABA from the cytosol into mitochondria [42].

Although the roles of many AATs were revealed in extensive studies of Arabidopsis, none of OsAATs was functionally characterized. Therefore, there is an urgent need for a thorough bioinformatics analysis of the OsAAT gene family. This study will provide comprehensive analyses of OsAATs, including the identification of all OsAAT family members, the phylogenetic relationships between OsAATs and AtAATs, as well as their expression profiling in different organs/tissues and under the treatments of abiotic stresses. These results will to a great extent benefit functional validation of the rice AAT genes and broaden our understandings of the roles of plant AATs.

## Results

### Identification of AAT Genes in Rice Genome

To explore the entire gene family of AATs in rice, through two domain searches (PF01490 and PF00324) at MSU-RGAP (MSU-Rice Genome Annotation Project) database (http://rice.plantbiology.msu.edu/analyses_search_domain.shtml), we obtained 84 and 47 sequences, respectively; while by the InterPro of European Bioinformatics Institute database, 308 protein sequences of OsAATs from japonica were deposited. In the Rice Genome Express Database, using keyword searches of "amino acid transporter" and "amino acid permease", 45 and 31 genes were identified, respectively. Sequences from these hits were used as queries employing BLAST algorithms to search against rice genome in the databases of both MSU-RGAP and NCBI. By removal of the sequence redundancies and alternative splice forms of the same gene, we initially identified 87 putative AAT genes in rice. All candidate AAT sequences were subjected to InterProScan for searching the presences of AAT domains. Four candidates were excluded from further analysis because they only contained short and incomplete AAT domains. Taken together, a total of 85 AATs were identified in rice. Meanwhile, similar methods were used to identify family members of AATs in Arabidopsis, and a total of 63 AtAATs were subsequently discovered. The detailed information about gene locus, FL-cDNA, ORF length for each OsAAT genes and characteristics of corresponding proteins are listed in Table 1, and those of AtAATs are showed in Table S1.

The numbers and positions of exons and introns were determined through the comparison of full-length cDNA sequences and the corresponding genomic DNA sequences of each OsAAT gene by using GSDS (http://gsds.cbi.pku.edu.cn/). Introns are absent in nine coding sequences of OsAAT genes, and the number of introns in other coding sequences range from one to thirteen (Figure S1). In the same subfamily, most members share similar intron/exon structures and gene lengths. For example, the three members of the OsProT subfamily have six introns and seven exons, and are nearly 4000 bp in length. The putative transmembrane (TM) regions in OsAATs were predicted by TMHMM Server v2.0 (http://www.cbs.dtu.dk/services/TMHMM/). The number of TM regions in most OsAATs ranges from eight to thirteen (Figure S2), and OsAATs of the same subfamily have similar number of TM regions, such as 11 TMs in LHT, 14 TMs in CAT, and 13 TMs in ACT. These observations revealed that members of the same subfamily were very conservative in structure.

### Chromosomal Localization and Gene Duplication

To investigate the relationship between the genetic divergence and gene duplication within the AAT family in rice, the chromosomal locations of OsAAT genes were determined based on the coordinates of RGAP loci (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/#search). OsAAT genes are distributed in all of rice 12 chromosomes. The densities of OsAAT genes are relatively higher in specific chromosomal regions, such as the bottom of chromosome 1 and 4, and the top and bottom of chromosome 2 and 12. In contrast, several large chromosomal regions are devoid of OsAAT genes, such as in the top parts of chromosomes 4 and 9, and in the bottom of chromosomes 7 and 11. The maximum members (19) of OsAAT genes are located in chromosome 1, followed by 11 genes in chromosome 12 and 10 genes in chromosome 4. In addition, nine genes each are located on chromosome 2, 3, and 6; four genes each on chromosome 5 and 11, three genes each on chromosome 7, 8, and 10. Nevertheless, a unique gene is localized on chromosome 9 (Figure 1). Similarly, the analysis of chromosomal location and gene duplication of 63 AtAATs was also performed in Arabidopsis (Figure S3).

Among the rice 85 OsAAT genes, 55.29% (47 of 85) come from the duplication events, including 24 gene segmental duplication and 23 gene tandem duplication. The 24 (12 pairs) OsAAT genes could be assigned to rice segmental duplication blocks based upon the analysis of the MSU-RGAP segmental duplication database. Four pairs of OsCATs, two pairs each of OsAAPs, OsANTs and OsATLs, and one pair each of OsGATs and OsAUXs are located on the duplicated segmental regions of rice chromosomes mapped by TIGR when the maximal length between collinear gene pairs is 500 kb. All of these duplicated genes exhibit high sequence similarity in both transmembrane regions and other domains. According to the criterion of separation by less than 5 intervening genes and ≥50% similarity at protein level, a total of 23 genes were found to be tandemly duplicated, falling into nine groups. Six groups comprised 2 genes and two groups comprised 3 genes in each group, and one group comprised 5 genes. The tandemly duplicated genes are only localized in five chromosomes, 3 groups on chromosome 1, 2 groups each on chromosome 6 and 12, and

**Table 1.** The general information and sequence characterization of 85 *OsAAT* genes.

| S.N. | Gene[a] | Accession Number | | ORF[d] (bp) | Protein[e] | | | TM[f] | Expression[g] |
|------|---------|------------------|--|-------------|------------|--|--|-------|---------------|
| | | RGAP Locus[b] | KOME[c] | | Size (aa) | MW(D) | pI | | |
| | AAP group | | | | | | | | |
| 1 | *OsAAP1* | LOC_Os07g04180 | AK120257 | 1464 | 487 | 52864 | 8.66 | 9 | A B C D |
| 2 | *OsAAP2* | LOC_ Os06g12330 | AK106762 | 1455 | 484 | 51898.8 | 8.32 | 11 | A B C D |
| 3 | *OsAAP3* | LOC_Os06g36180 | AK102763 | 1464 | 487 | 52783.8 | 7.93 | 11 | A B C D |
| 4 | *OsAAP4* | LOC_Os12g09300 | AK069508 | 1407 | 468 | 50891.2 | 8.27 | 9 | A B C D |
| 5 | *OsAAP5* | LOC_Os01g65660 | AK073884 | 1398 | 465 | 50002.2 | 8.77 | 11 | A B C D |
| 6 | *OsAAP6* | LOC_Os01g65670 | AK121636 | 1401 | 466 | 50085.5 | 8.68 | 11 | A B C D |
| 7 | *OsAAP7* | LOC_Os05g34980 | AK065891 | 1491 | 496 | 53513.2 | 8.02 | 9 | A B C D |
| 8 | *OsAAP8* | LOC_Os01g66010 | AK059325 | 1467 | 488 | 52871.4 | 8.28 | 9 | A B C D |
| 9 | *OsAAP9* | LOC_Os02g01210 | AK060685 | 1557 | 518 | 57206.4 | 8.63 | 9 | A B C D |
| 10 | *OsAAP10* | LOC_Os02g49060 | AK070030 | 1410 | 469 | 50249.8 | 8.03 | 10 | A B C D |
| 11 | *OsAAP11* | LOC_Os11g09020 | AK068003 | 1431 | 476 | 51346.8 | 8.77 | 9 | A B C D |
| 12 | *OsAAP12* | LOC_Os12g09320 | NA | 1407 | 468 | 50067.7 | 8.04 | 9 | C D |
| 13 | *OsAAP13* | LOC_Os04g39489 | AK071044 | 1401 | 466 | 50746.9 | 8.15 | 9 | A B C D |
| 14 | *OsAAP14* | LOC_Os04g56470 | NA | 1410 | 469 | 51377.8 | 8.64 | 9 | B C D |
| 15 | *OsAAP15* | LOC_Os12g08130 | AK061470 | 1428 | 475 | 51063.2 | 8.73 | 9 | A B C D |
| 16 | *OsAAP16* | LOC_Os12g08090 | AK061448 | 1428 | 475 | 51091.3 | 8.73 | 9 | A B C D |
| 17 | *OsAAP17* | LOC_Os06g12350 | AK106814 | 1524 | 507 | 53441.6 | 9.55 | 9 | A B C D |
| 18 | *OsAAP18* | LOC_Os06g36210 | AK071510 | 1425 | 474 | 50720.6 | 8.30 | 6 | A B C D |
| 19 | *OsAAP19* | LOC_Os04g41350 | NA | 1236 | 411 | 43154.1 | 8.47 | 7 | C D |
| | LHT group | | | | | | | | |
| 20 | *OsLHT1* | LOC_Os08g03350 | AK102015 | 1344 | 447 | 49830.8 | 9.45 | 10 | A B C D |
| 21 | *OsLHT2* | LOC_Os12g14100 | AK070297 | 1341 | 446 | 48941.5 | 8.77 | 11 | A B C |
| 22 | *OsLHT3* | LOC_Os05g14820 | NA | 1371 | 456 | 49939.7 | 9.12 | 11 | C D |
| 23 | *OsLHT4* | LOC_Os04g38860 | NA | 1335 | 444 | 47795.9 | 9.00 | 7 | B C D |
| 24 | *OsLHT5* | LOC_Os04g47420 | AK060598 | 1539 | 512 | 55030.7 | 9.11 | 11 | A B C D |
| 25 | *OsLHT6* | LOC_Os12g30040 | AK100852 | 1527 | 508 | 54950.1 | 8.97 | 11 | A B C D |
| | GAT group | | | | | | | | |
| 26 | *OsGAT1* | LOC_Os05g50920 | AK106883 | 1446 | 481 | 51041.1 | 9.43 | 10 | A B C D |
| 27 | *OsGAT2* | LOC_Os01g43320 | NA | 1365 | 454 | 48525.1 | 9.02 | 11 | B C D |
| 28 | *OsGAT3* | LOC_Os01g63854 | AK103684 | 1374 | 457 | 48769.2 | 8.73 | 9 | A B C D |
| 29 | *OsGAT4* | LOC_Os10g27980 | AK119782 | 1329 | 442 | 47984.5 | 8.92 | 10 | A B C D |
| | ProT group | | | | | | | | |
| 30 | *OsProT1* | LOC_Os01g68050 | NA | 1344 | 447 | 49024.9 | 9.40 | 10 | B C D |
| 31 | *OsProT2* | LOC_Os03g44230 | AK067118 | 1422 | 473 | 52053.6 | 7.90 | 11 | A B C D |
| 32 | *OsProT3* | LOC_Os07g01090 | AK066298 | 1305 | 434 | 47663.8 | 9.51 | 11 | A B C D |
| | AUX group | | | | | | | | |
| 33 | *OsAUX1* | LOC_Os01g63770 | AK100090 | 1479 | 492 | 54762 | 8.15 | 10 | A B C D |
| 34 | *OsAUX2* | LOC_Os05g37470 | AK111849 | 1512 | 503 | 55662.9 | 8.65 | 11 | A B C D |
| 35 | *OsAUX3* | LOC_Os03g14080 | AK103524 | 1575 | 524 | 58083.4 | 9.21 | 10 | A B C D |
| 36 | *OsAUX4* | LOC_Os10g05690 | AK102295 | 1644 | 547 | 59846.2 | 8.64 | 10 | A B C D |
| 37 | *OsAUX5* | LOC_Os11g06820 | NA | 1443 | 480 | 52956.5 | 9.33 | 9 | B C D |
| | ANT group | | | | | | | | |
| 38 | *OsANT1* | LOC_Os07g12770 | AK105808 | 1275 | 424 | 45712.3 | 7.01 | 11 | A B C D |
| 39 | *OsANT2* | LOC_Os03g60260 | AK121940 | 1257 | 418 | 43571.8 | 7.79 | 9 | A B C D |
| 40 | *OsANT3* | LOC_Os02g44980 | AK100650 | 1269 | 422 | 44846.9 | 7.53 | 11 | A B C D |
| 41 | *OsANT4* | LOC_Os04g47780 | AK058888 | 1278 | 425 | 44788.8 | 7.84 | 9 | A B C D |
| | ATLa group | | | | | | | | |

**Table 1.** Cont.

| S.N. | Gene[a] | Accession Number | | ORF[d] (bp) | Protein[e] | | | TM[f] | Expression[g] |
|------|---------|------------------|------|-------------|------------|------|------|-------|---------------|
| | | RGAP Locus[b] | KOME[c] | | Size (aa) | MW(D) | pI | | |
| 42 | OsATL1 | LOC_Os06g43700 | AK070101 | 1461 | 486 | 51755.2 | 7.55 | 10 | A B C D |
| 43 | OsATL2 | LOC_Os09g26290 | NA | 927 | 308 | 32736.3 | 8.61 | 4 | C D |
| 44 | OsATL3 | LOC_Os02g49510 | AK069154 | 1347 | 448 | 48108.2 | 7.18 | 11 | A B C D |
| 45 | OsATL4 | LOC_Os06g16420 | AK120497 | 1347 | 448 | 48121.5 | 7.30 | 11 | A B C D |
| 46 | OsATL5 | LOC_Os06g42720 | AK101315 | 1377 | 458 | 49977.6 | 6.42 | 10 | A B C D |
| 47 | OsATL6 | LOC_Os02g09810 | AK069748 | 1380 | 459 | 50149.7 | 6.45 | 10 | A B C D |
| 48 | OsATL7 | LOC_Os01g61044 | AK102220 | 1380 | 459 | 47870.2 | 9.80 | 11 | A B C D |
| | ATLb group | | | | | | | | |
| 49 | OsATL8 | LOC_Os11g19240 | NA | 1452 | 483 | 50225.9 | 9.08 | 11 | C D |
| 50 | OsATL9 | LOC_Os02g54730 | AK066747 | 1647 | 548 | 59332.3 | 5.07 | 10 | A B C D |
| 51 | OsATL10 | LOC_Os12g38570 | AK069006 | 1785 | 594 | 63407.2 | 4.63 | 10 | A B C D |
| 52 | OsATL11 | LOC_Os02g01100 | AK074023 | 1725 | 574 | 62911.4 | 5.92 | 9 | A B C D |
| 53 | OsATL12 | LOC_Os06g12320 | AK107472 | 1242 | 413 | 43792.8 | 9.41 | 11 | A B C D |
| 54 | OsATL13 | LOC_Os04g38680 | AK106202 | 1368 | 455 | 47994.6 | 8.30 | 10 | A B C D |
| 55 | OsATL14 | LOC_Os04g38660 | NA | 690 | 229 | 24225.6 | 9.39 | 6 | C |
| 56 | OsATL15 | LOC_Os01g41420 | NA | 1896 | 631 | 67357.3 | 9.70 | 10 | B C D |
| 57 | OsATL16 | LOC_Os01g41400 | NA | 1332 | 443 | 47386.9 | 8.75 | 10 | C D |
| 58 | OsATL17 | LOC_Os01g40410 | NA | 1383 | 460 | 49420.1 | 5.89 | 10 | C |
| | CAT group | | | | | | | | |
| 59 | OsCAT1 | LOC_Os01g11160 | AK099094 | 1851 | 616 | 65510.9 | 8.13 | 14 | A B C D |
| 61 | OsCAT2 | LOC_Os02g43860 | AK101068 | 1818 | 605 | 64436.6 | 7.57 | 13 | A B C D |
| 60 | OsCAT3 | LOC_Os03g43970 | NA | 1329 | 442 | 46938 | 8.54 | 12 | B |
| 62 | OsCAT4 | LOC_Os03g45170 | AK066436 | 1920 | 639 | 68202.9 | 5.74 | 14 | A B C D |
| 63 | OsCAT5 | LOC_Os04g45950 | AK064289 | 1686 | 561 | 60780.4 | 8.3299 | 13 | A B C D |
| 64 | OsCAT6 | LOC_Os06g34830 | AK063382 | 1791 | 596 | 61918.3 | 8.42 | 14 | A B C D |
| 65 | OsCAT7 | LOC_Os10g30090 | AK100205 | 1869 | 622 | 66053.4 | 6.73 | 14 | A B C D |
| 66 | OsCAT8 | LOC_Os11g05690 | NA | 1413 | 470 | 49758.9 | 5.96 | 12 | B D |
| 67 | OsCAT9 | LOC_Os12g06060 | NA | 1608 | 535 | 56371.8 | 7.26 | 13 | C D |
| 68 | OsCAT10 | LOC_Os12g41890 | NA | 1806 | 601 | 62597.4 | 8.44 | 14 | D |
| 69 | OsCAT11 | LOC_Os12g42850 | AK064822 | 1866 | 621 | 66108 | 5.80 | 14 | A B C D |
| | ACT group | | | | | | | | |
| 70 | OsBAT1 | LOC_Os01g42234 | AK071623 | 1599 | 532 | 57024.1 | 8.51 | 12 | A B C D |
| 71 | OsBAT2 | LOC_Os01g71700 | NA | 1089 | 521 | 38637.4 | 9.055 | 12 | B C D |
| 72 | OsBAT3 | LOC_Os01g71710 | NA | 1566 | 362 | 55915.2 | 8.8742 | 9 | C D |
| 73 | OsBAT4 | LOC_Os01g71720 | AK065371 | 1578 | 525 | 55991.8 | 7.76 | 13 | A B C D |
| 74 | OsBAT5 | LOC_Os01g71740 | NA | 1554 | 517 | 55683.9 | 8.70 | 12 | B C D |
| 75 | OsBAT6 | LOC_Os01g71760 | NA | 1527 | 508 | 54464.5 | 9.00 | 12 | C D |
| 76 | OsBAT7 | LOC_Os04g35540 | AK072850 | 1593 | 530 | 57764.3 | 8.08 | 11 | A B C D |
| | PHS group | | | | | | | | |
| 77 | OsLAT1 | LOC_Os02g47210 | AK068055 | 1695 | 564 | 60174.1 | 6.18 | 12 | A B C D |
| 78 | OsLAT2 | LOC_Os03g25840 | NA | 993 | 330 | 35288.6 | 8.98 | 3 | D |
| 79 | OsLAT3 | LOC_Os03g25869 | AK072316 | 1644 | 547 | 59972.5 | 12.42 | 5 | A B C D |
| 80 | OsLAT4 | LOC_Os03g25920 | AK107064 | 1500 | 499 | 51811.2 | 8.96 | 10 | A B C D |
| 81 | OsLAT5 | LOC_Os03g37984 | AK071314 | 1653 | 550 | 60375 | 7.27 | 10 | A B C D |
| 82 | OsLAT6 | LOC_Os08g41370 | NA | 579 | 192 | 21285.3 | 8.97 | 5 | / |
| 83 | OsLAT7 | LOC_Os12g39080 | AK099986 | 1344 | 447 | 48989.6 | 12.27 | 5 | A B C D |
| 84 | OsLAT8 | LOC_Os01g19850 | NA | 2391 | 796 | 88296.8 | 7.9546 | 7 | B C D |

**Table 1.** Cont.

| S.N. | Gene[a] | Accession Number | | ORF[d] (bp) | Protein[e] | | | TM[f] | Expression[g] |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | RGAP Locus[b] | KOME[c] | | Size (aa) | MW(D) | pl | | |
| 85 | OsLAT9 | LOC_Os08g23440 | NA | 2970 | 989 | 108454 | 6.2146 | 11 | B C D |

[a]Systematic designation given to rice *AATs* in this study.
[b]Locus identity number of *OsAATs* assigned by RGAP.
[c]Full-length cDNA accession number of *OsAATs* obtained from KOME.
[d]Length of the open reading frame for *OsAATs*.
[e]Protein characterization of OsAATs obtained from RGAP.
[f]Number of transmembrane segments possessed by OsAATs, predicted by the TMHMM Server v2.0.
[g]Evidence for gene expression from (A) full-length cDNA, (B) ESTs, (C) microarray data, (D) massively parallel signature sequencing (MPSS).
S.N., serial number; ORF, open reading frame; bp, base pair; aa, amino acids; MW, molecular weight; pl, isoelectric point; TM, transmembrane; NA, not available.
doi:10.1371/journal.pone.0049210.t001

one group each on chromosome 3 and 4 (Figure 1). Moreover, all of the proteins of the duplicated genes have relatively high sequence similarity. For example, OsAUX1 and OsAUX2 from segmental duplication are 82% similarity, and OsAAP3 and OsAAP18 from tandem duplication are 70% similarity. These results suggested that large-scale segmental and tandem duplication events played a significant role in the expansion of the *OsAAT* family.

### Phylogenetic Analysis and Multiple Sequence Alignment

In order to evaluate the evolutionary relationship among the 85 members of OsAATs, phylogenetic analysis was performed based on the alignment of full-length amino acid sequences of the 85 AAT proteins. Eleven distinct clades supported with high bootstrap values by the neighbor-joining method were identified. Additionally, baysian inference was also used to construct the phylogeny by using MrBayes program (Figure S4). Based on the domain composition and phylogenetic relationship, the OsAATs can be divided into two main families similar to that in *Arabidopsis*, including the AAAP and APC family (Figure 2A). The AAAP family consists of 58 OsAATs, including eight distinct subfamilies: amino acid permeases (AAPs), lysine, histidine transporters (LHTs), proline transporters (ProTs), GABA transporters (GATs), auxin transporters (AUXs), aromatic and neutral amino acid transporters (ANTs) and amino acid transporter-like (ATL) subfamilies. So far, the characterization of these genes in the ATL subfamilies has not been reported in *Arabidopsis* and other plant species. ATL subfamilies consist of two phylogenetic clades that are named as ATLa and ATLb, respectively. The five auxin transporters in OsAUXs subfamily were initially described as auxin influx-like carriers (OsLAX1-5) [43,44]. Since the abbreviation LAX in rice was also used for the lax panicle and *OsLAX1* and *OsLAX2* were functionally characterized to regulate the formation of axillary meristems [45,46], the five auxin transporters in rice were renamed to OsAUX1-5. The APC family is comprised of 27 OsAATs and subdivided into three distinct subfamilies, including the cationic amino acid transporters (CATs), the amino acid/choline transporters (ACTs) and the polyamine H⁺-symporters (PHSs).

In order to identify orthologous genes between rice and *Arabidopsis*, a combined phylogenetic tree with OsAATs and AtAATs was also established by using the neighbor-joining method (Figure S5). Similar subfamilies were formed as compared to the tree of OsAATs. Each clade of distinct subfamily consists of AATs from both rice and *Arabidopsis*, indicating the main characteristics formation of AAT family in rice and *Arabidopsis* before the split of monocotyledonous and dicotyledonous plants.

Moreover, the difference in the total number of *OsAATs* and *AtAATs* is mainly due to the variation in the number of genes in *AAP* subfamily and APC family, there were 19 AAPs in rice and 8 in *Arabidopsis*; 27 APCs in rice and 15 in *Arabidopsis*.

Moreover, the MEME motif search tool was employed to identify the motifs shared in the OsAATs. Subsequently, 20 motif sequences were identified and shown in Table S2. Besides, the distributions of these motifs in OsAATs were shown in Figure 2B. Several motifs were widespread among OsAATs in AAAP family (e.g. motif 2 and 6). In contrast, other motifs were specific to only one or two subfamilies. For example, motifs 1 and 7 were specific to subfamily AAPs, and motifs 8 and 5 were specific to AUXs and ACTs, respectively, while motif 12 and 17 were found in subfamily CAT and ATLa, respectively. However, motif 15 was present in the AAP, GAT, ATLb and ANT subfamilies. In addition, motif 11 exclusively appeared in the ATLa, CAT and PHS subfamilies.

The alignments of the OsAATs' amino acid sequences illustrated that most of TM regions in the same subfamily were very conserved. In addition, several TM regions of different members varied insignificantly both in length and amino acid composition. The alignment of the OsLHT members was shown in Figure 3 as an example. The overall identities of the protein sequences of these genes are 55.75%. There are five conserved motifs in OsLHTs, including motif 2, 6, 10, 4 and 18. Motif 2 was found to be located in the first and second TM region, and motif 6 comprised the fourth and fifth TM region. Motif 10 consisted in the sixth TM region. Motif 4 was located at the eighth TM region and extended into the following sequences before the ninth TM region. Motif 18 was located in the tenth and eleventh TM region.

### Expression Analysis of *OsAAT* Genes in Various Organs at Different Developmental Stages

Several approaches were employed to analyze the expression patterns of the *OsAAT* genes in different tissues and organs, including expressed sequence tag (EST) profiles, microarray data and massively parallel signature sequencing (MPSS) tags.

In analysis of EST profiles (http://www.ncbi.nlm.nih.gov/unigene/), the availability of any corresponding full-length cDNA (FL-cDNA) and ? or ESTs in UniGene database at NCBI was searched. Seventy-one of 85(83.53%) *OsAAT* genes have at least one corresponding FL-cDNA and ? or EST sequence (Table S3); fifty-nine *OsAAT* genes of them (69.41%) have both FL-cDNA and EST evidence, whereas twelve genes (14.12%) have only EST evidence (Table 1), indicating that most of these genes are expressed. Various *OsAAT* genes show high expression abundance in stem, root, leaf, panicle, and seed, several of which have tissue-
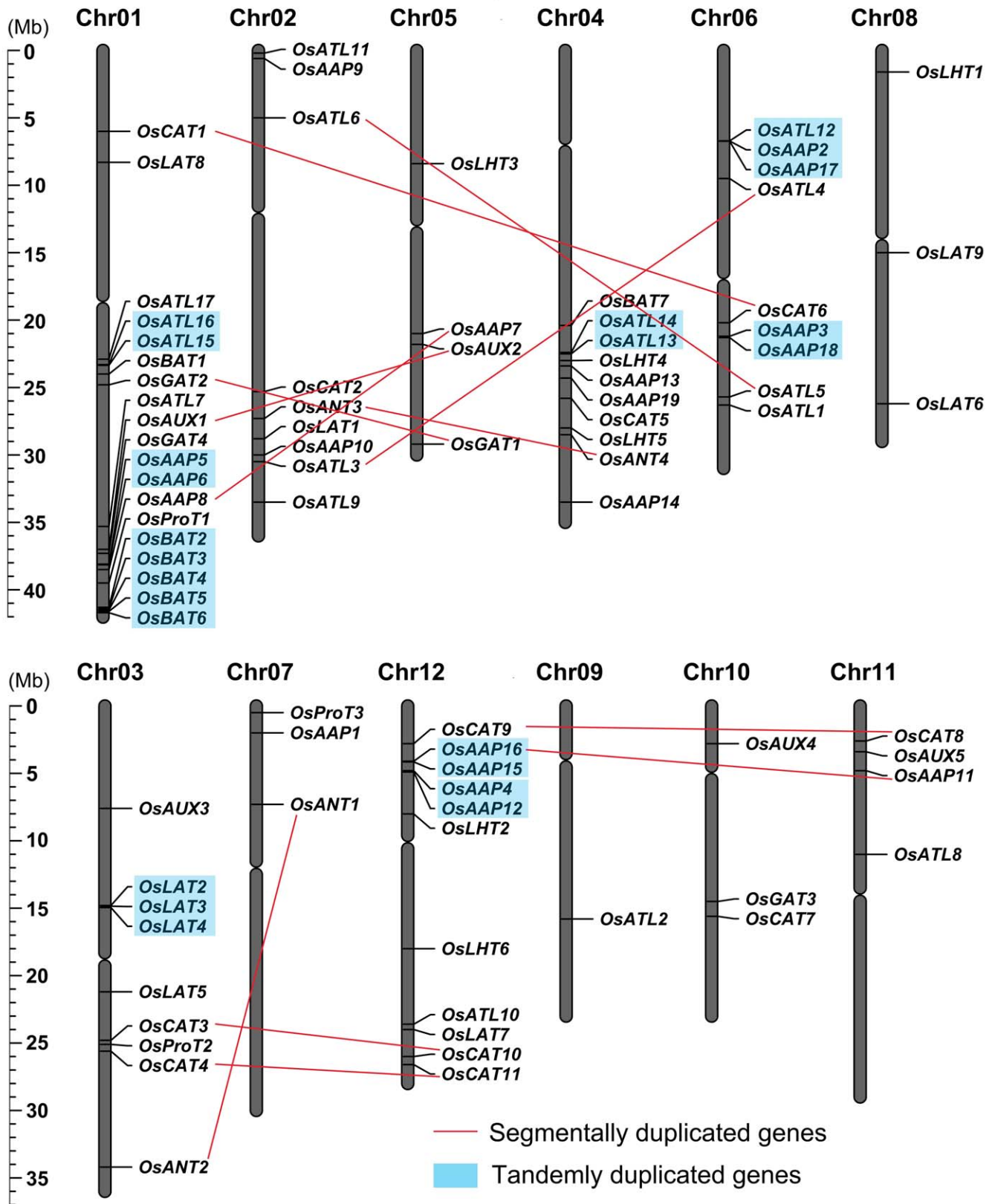
**Figure 1. Chromosomal localization and gene duplication events of *OsAAT* genes.** Respective chromosome numbers are indicated at the top of each bar. The scale on the left is in megabases (Mb). The cleavages on the chromosomes (vertical bars) indicate the position of centromeres. The chromosome order is arranged to bring duplicated regions in the vicinity.
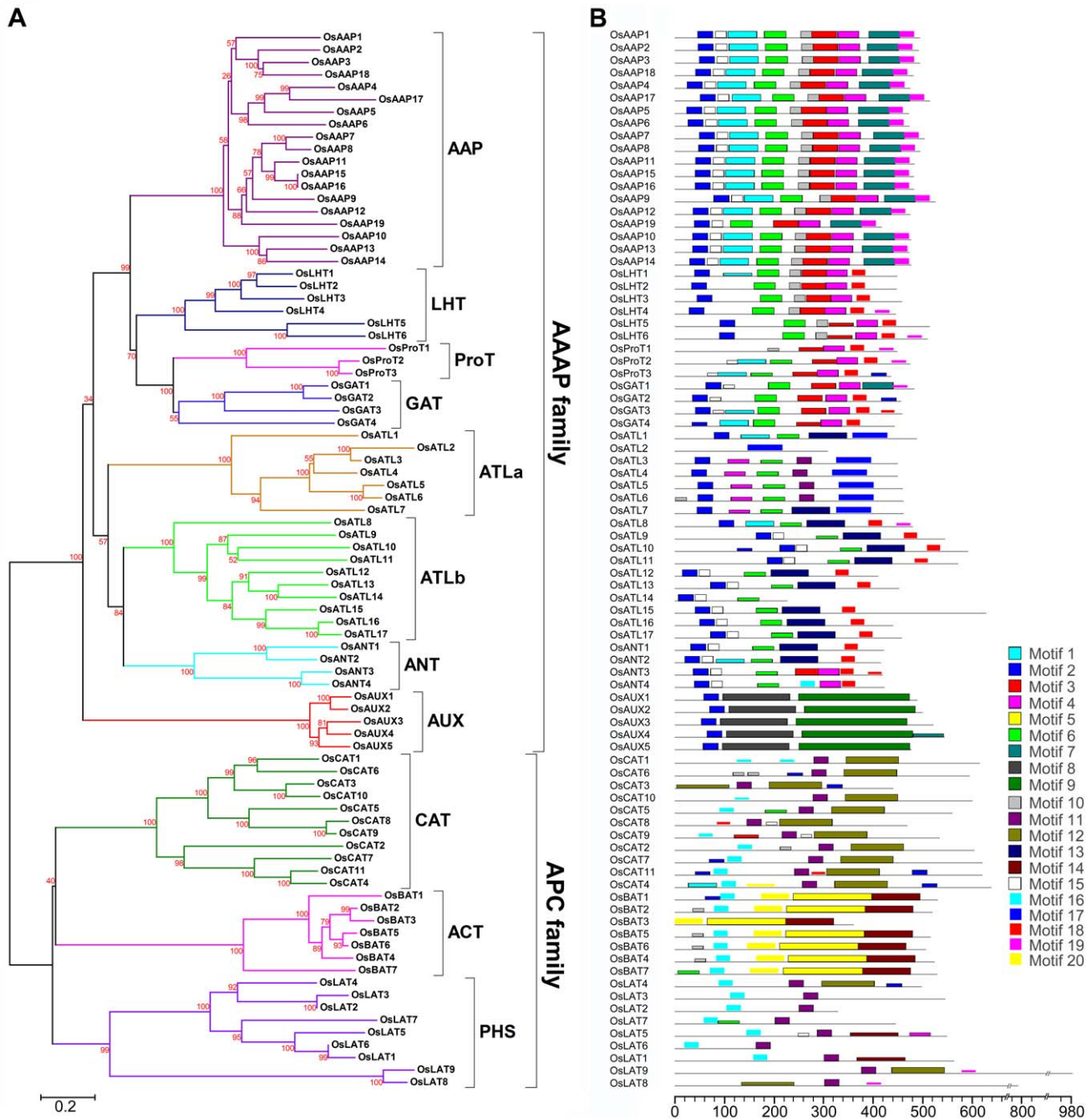doi:10.1371/journal.pone.0049210.g001

**Figure 2. Phylogenetic relationship and protein motifs of OsAATs.** (A) Phylogenetic tree of OsAATs constructed by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each node. Scale bar represents 0.2 amino acid substitution per site. The proteins on the tree can be divided into eleven distinct subfamilies. Subfamilies ATL are further divided into two groups (ATL a and ATL b). The branches of different subfamilies are marked with different colors. (B) Protein motifs of OsAATs. Each colored box represents a specific motif in the protein identified using the MEME motif search tool. The order of the motifs corresponds to their position within individual protein sequences.
doi:10.1371/journal.pone.0049210.g002

specific or abundant expression patterns: *OsAAP6*, *OsAAP14*, and *OsAUX3* in callus; *OsLHT2*, *OsATL10* and *OsLAT5* in flower; *OsATL15*, *OsLAT7* and *OsCAT11* in leaf; *OsAAP1*, *OsAAP15* and *OsAAP17* in panicle; *OsLHT1*, *OsAUX1* and *OsBAT7* in root; *OsBAT5* in seed and *OsBAT4* in stem (Table S3).

Microarray data (http://signal.salk.edu/cgi-bin/RiceGE) of various tissues during vegetative and reproductive developmental stages of rice was used for analyzing the expression profiles of

*OsAAT* genes, including young root (YR), mature leaf (ML), young leaf (YL), shoot apical meristem (SAM), panicles (P1–P6), and seed (S1–S5) development. At least one probe could be found on Affymetrix rice whole-genome array platform (GPL2025) for 80 of 85 *OsAAT* genes. A hierarchical cluster display generated from the average log signal values for the 80 *OsAAT* genes in selected organs indicates the differential expression patterns of these genes (Table S4). The expression patterns of 80 *OsAAT* genes can be divided

```
OsLHT1  ..................................................MGTQVADNYPPAKDGRSA  18
OsLHT2  .......................................................MAPPSAMNTSRA  12
OsLHT3  ....................................................MSATEVMEECTETARERRE  19
OsLHT4  ...............................................................MVT   3
OsLHT5  ..............MSSEVTSVPPTPTPPPVSTPPSQIQSPAAPASSRASPLRGMGTPNIASPVRKAVASVSGYLGEVG  65
OsLHT6  MAMAVEGAGAGVVVSEPEVVSIPPTPRGMSTPDGTATPPPAAGRGA...........ATPARRVVEGLRGYLEEVG  65
```

                                    TM1                          TM2
```
OsLHT1  QEKAI....DDWLPITSSRNAKWWYSAFFNVTAMVGAGVLSLFYAMSELGWGPGIAVLILSWIITLYTLWQMVEMHE  91
OsLHT2  EEKAI....DDWLPITSSRNAKWWYSAFFNVTAMVGAGVLSLFFAMSELGWGPGVAAMIMSWIITLYTLWQMVEMHE  85
OsLHT3  EERLRNVNLDDWLPITSSRTAKWYYSAFFNVTAMVGAGVLGLFAMSQLGWPTGVAAIASSFAITLYTLWQLVELHE  96
OsLHT4  SSVLPKVVDDAGEGEANFRRAKWWYATFFSVTAMVGAGVLSLFYAMAHLGWGPGTAALVVSWGMTLYTLRLLIELHE  80
OsLHT5  QMTRLADPRDAWLPITESRSGNAYYAAFFSLSSGIGFQALVLFVAFSLLGWTWAIICLVAFAWQLYTLWLLVKLHE  142
OsLHT6  HLTRLN.PQDAWLPITESRSGNARYAAFFTLNAGIGFQALVLILAFPALGWSWGIISLTVAYFWQLYTLWILVKLHE  141
```
                                    Motif 2
                                    TM3                          TM4
```
OsLHT1  MVP.GKRFDRYHELGQH..AFGEKIGLWIVVPQQLVVEVGVNIVYMVTGGKSLKKFHD.VLCEGHGCKNIKLTYFIM  164
OsLHT2  CVP.GRRFDRYHELGQH..AFGDKIGLWIVVPQQLVVEVGVCIVYMVTGGKSLKKFHD..LVAPPSAPPIRTSYFIV  157
OsLHT3  PAP.GGGKRFDRYHELGQAFGRRIGVCLIVPLQLIVQVGTDIVYMVTGGQTLKKFVE..LACDGR.CADIRLTFYIM  170
OsLHT4  CVP.GVRFDRYDLGAH..AIGPRIGPWLVVPQQLIVQLGCDVVYMVIGGKCLMKFAESVSSWSRAPQLHHQSYWIC  154
OsLHT5  PVAGGTRYSRYMYLATT..VFGEKWGKILALLPVMYLSAGTCTALIIVGGGSMKLLFNIACGEVCLARPLTTVEWYI  217
OsLHT6  AVP.GRRYNRYVELAQA..AFGEKIGMWLSLFPTIYLSAGSATALIILVGGETMKLFYQIVCGPICSPSPISTIEWYI  215
```
                                    TM5
```
OsLHT1  IFASVHFVLSCLPNFNSISGVSLAAAVMSLSYSTIAWGASVDKGKV...AD......VDYHLRATTISTGKV.FGFFS  231
OsLHT2  IFGCLHLVLSCLPNFNSISGVSLAAAVMSLSYSTIAWAASLHHPNH..NNGAAAGGVDYSLTEATPAGRT.FNFLS  230
OsLHT3  MFASAQFVLSCPNFNSISAVSAAAAMSLCYSMIAFFASVLKAHPAAAAA..VDYGFKATTAAGRV.FGAFN  240
OsLHT4  IFGASQFILSCLPSLDSITAVSLAAAMSVGYSTISWAACLARGTP..AAAEGGGGGVSYAYKDGTAADSV.FRVCS  228
OsLHT5  VFVCVAALLSCLPNLNSIAGVSLVGATAAVAYCTMIWVVSVAKGRV...AG......VSYDPVRTTDEEDGAIGILN  285
OsLHT6  VFTSLAIVLSCLPNLNSIAGVSLIGGTTAITYCTMSWVLSVSQPQP...PT......VSYEPQAYISLGSSLFSTLN  283
```
                                    Motif 6
        TM6                                      TM7
```
OsLHT1  ALGDVAFAYAGHNVVLEIQATIPSTPEKESKKEMWKGVVVAYIIVALCYFFVALVGYWAFGNHVDDNILITLSR...  305
OsLHT2  ALGDVAFAYAGHNVVLEIQATIPSTAERESKGEMWRGVVIAYGVVAVCYLFVAFAGYYVFGNAVDDNVLITLER...  304
OsLHT3  ALGAVSFAFAGHNVVLEIQATIPSTPEREBKREMWRGVVVAVVALCYFTVAFGGYHAFGNAVAPNVLISLEK...  314
OsLHT4  ALGQVAFAYAGHGVVLEIQATIPSTPTKESRGAMWKGAVAAYLVTAICYFFVAIAGYWAFGRDVSDNVLVALRR...  302
OsLHT5  GLGIIAFAFRGHNLVLEIQATIYPSTKHFSHVEMWKGVKAAYVIIAICLYFVAVGGFWAYGDQIPPNGILSALYKFH  362
OsLHT6  ALGIIAFAFRGHNLALEIQATIPSTFKHFAHVEMWRGAKVAYALIAMCLFFVAIGGYWAYGNMVPQGGMLTALYAFH  360
```
        Motif10
                    TM8                                    TM9
```
OsLHT1  ....PKWLIALANMMVVIHVIGSYQIYAMEVFDMIBTVLVKKLRFPPGLTIRLIARTLYVAFTMFIAITFPFFGGLL  378
OsLHT2  ....PAWLIAAANMFVVVHVVGSYQIYAMEVFDMIBTFLVKKLRFKPGMPIRLIARSLYVLFTMVAIAVPFFGGIL  377
OsLHT3  ....PRWLVAAANLMVVVHVIGAYQVYAMEVFDMIBTVLAKKLHLRPGLPIRVTARSAYVALTMFIGITFPFFDGLL  387
OsLHT4  ....PPWLVAANMMVVVHVLGSYQVYAMEIFETLETILITRIRLPPGALIRLVARSAYVAFTLFVAVTFPFFGDLL  375
OsLHT5  SQDVSRVVLGTATLLVIVNCLTTYQIYAMEVFDNMBTGYVHKKNRPCPWWMRAGFRALFGAINLLIAVALPFLSEIA  439
OsLHT6  SHDIPRGLLAATFLIVVFNCLSSFQIYSMEVFDSFPAAYTGRTNRPCSVWVRSGFRVFYGFLSLFISVAIPFLSSIA  437
```
                    Motif 4                                Motif 18
        TM10                          TM11
```
OsLHT1  GFFGGFAFAPTTYFIPCIMWLAIYKERRFSLSWFTNWICIILGVMLMILSPIGGLRQIIIDAKTYKFYS....  447
OsLHT2  GFFGGFGFAPTTYFIPCIMWLSIMKEKRFGLSWCINWFCIIIGVLLSVFAPIGGLRSIIVNAQSYKFFS....  446
OsLHT3  GFFGGFGFAPTTYFIPCIIWLIMRKEAKYSLSWFANWGCIVVGMLLMLVSPIGGLRQIIILDASKYKFYS....  456
OsLHT4  GFFGGFGFTPTSYFIPCIIWLKIKKEPRFSASWFANWGCIVVGVLLMIASTIGGLRSIIQDASTFQFYS....  444
OsLHT5  GLIGGISLIBVTLAYPCFMWVAIMREAKGTAMWYTNWGLGSLCMGLSFVLIVCNLWGLVEKGLHVKFFKPADF  511
OsLHT6  GLIGGLTLIBVTFAYPCFMWICIKKEERFSSGWYINWGLGLGTAFSLALCVGGVWSIISSGMKFKFFKPPS.  508
```

**Figure 3. Multiple sequence alignment and transmembrane region of OsAATs.** Identical (100%), conservative (75-99%) and block (50-74%) of similar amino acid residues are shaded in deep blue, cherry red and gray, respectively. The transmembrane regions are marked by blue lines. The conserved motifs 2, 6, 10, 4, 18 are orderly marked by red rectangles.
doi:10.1371/journal.pone.0049210.g003

into six major groups (Figure 4). Twelve *OsAAT* genes in group I show high expression levels in all examined organs, expect for five genes with relatively low expression in certain organs (*OsAAP16* and *OsLHT1* in SAM, *OsAAP15* in SAM and S5, *OsATL4* in SAM and P1–P3, and *OsAAP1* in YL). Group II includes 13 genes that show abundant expression level in vegetative organs. For example, four genes (*OsAUX2*, *OsAAP4*, *OsProT3* and *OsATL12*) display specifically or predominantly expression level in YR; *OsCAT2*, *OsAAP7* and *OsCAT4* were abundantly expressed in YR, ML and YL. Group III comprises 21 genes that show relatively high expression level in specific organs (*OsAUX3* in SAM, *OsBAT4* in YL and ML, *OsBAT1*, *OsGAT3*, *OsGAT4* and *OsLHT4* in YR, *OsCAT6*, *OsAAP18*, *OsLHT2*, *OsAAP17* and *OsATL10* in P6). Conversely, group IV contains 18 genes that show relatively low expression level in all examined organs. Group ? comprises 8 genes with preferential expression patterns in different stages of seed development, meanwhile, *OsProT2* and *OsCAT1* are highly expressed in ML, and *OsAAP13* and *OsLAT7* in YR. Group VI consists of 4 genes which show relatively high expression levels in several certain reproductive organs (*OsAUX4* and *OsLHT6* in SAM, *OsATL13* in P1–P4 and S4–S5, *OsANT1* in S4–S5).

In the mRNA MPSS database of rice (http://mpss.udel.edu/rice/), the expression of *OsAAT* genes were investigated. MPSS generates hundreds of thousands of molecules per reaction and provides a quantitative assessment of transcript abundance. MPSS signatures are available for the 80 genes in at least one of the libraries (Table S5), further indicating that most *OsAAT* genes are expressed. Differential expression abundances are displayed by number of tags (tpm, transcripts per million), being low in <50, moderate in between 50 tpm and 500 tpm, and strong in >500 tpm. A large percentage of *OsAAT* genes (47 of 80) have low expression level, 30 genes moderate level, and three genes (*OsAUX1*, *OsATL5* and *OsATL15*) high level (Table S5).

To validate the results of digital expression analysis, we detected the expression levels of several representative genes by real-time PCR analysis. The results show that they are in general agreement with the data of microarray and MPSS tags. For example, *OsAAP15*, *OsATL15*, *OsAUX4* and *OsAAP8* are significantly expressed in stems, meanwhile, *OsAAP15*, *OsATL15* and *OsAAP8* are also expressed in roots and leaves, and *OsAUX4* in root and in panicles at early stages of development (P1) (Figure 5A). *OsATL9*, *OsCAT1*, *OsBAT7*, *OsAAP5*, *OsANT1*, *OsATL6* and *OsAAP7* are predominantly or highly expressed in leaves (Figure 5B). *OsLHT6*, *OsAUX5* and *OsAAP11* are mainly expressed in panicles at early stages of development (P1), and *OsAUX5* is also highly expressed in roots (Figure 5C). Otherwise, *OsProT1*, *OsAUX1*, *OsAUX2*, *OsATL13*, *OsAAP4*, *OsANT3* and *OsATL1* are predominantly or highly expressed in panicles at late stages of development (P2), and *OsATL13* similarly in panicles at early stages of development (Figure 5D). *OsBAT1* and *OsAAP6* are highly expressed in roots and seeds, respectively. *OsAUX3* is predominantly expressed in stem and P1, but was not detected in either leaf or P2 (Figure 5E).

## Expression Regulation of *OsAAT* Genes under Abiotic Stresses

To determine the response of *OsAAT* genes to abiotic stress, the data of microarray (GSE6901) of 7-day-old seedlings subjected to the treatments of drought, salt and cold stresses were analyzed. It was revealed that a total of 21 genes were evidently down- or up-

regulated (<0.5 or >2) in at least one of the stress conditions examined as compared with the control (Figure 6A–G). The expression of three genes (*OsAAP15*, *OsATL6* and *OsANT3*) were up-regulated by all three stresses (Figure 6A), five genes (*OsATL13*, *OsAAP6*, *OsAAP11*, *OsAAP13* and *OsAAP5*) were up-regulated by drought and salt stresses (Figure 6B), and two genes (*OsGAT2* and *OsCAT6*) and three genes (*OsANT4*, *OsBAT7* and *OsATL11*) were specifically up-regulated by drought and salt stress, respectively (Figure 6C and D). However, four genes (*OsAUX1*, *OsAAP4*, *OsBAT4* and *OsAAP8*) were down-regulated by all three stresses (Figure 6E), three genes (*OsProT3*, *OsAUX2* and *OsLAT7*) were down-regulated by drought and salt stresses (Figure 6F), and one gene (*OsATL9*) was down -regulated by drought stress (Figure 6G). By using real-time PCR analysis, we validated the expression levels of 9 representative *OsAAT* genes in 7-day-old seedling under three stress conditions. It was found that the results were in very well agreement with the microarray data (Figure 6 H, I, J and K), indicating that *OsAAT* genes might participate in abiotic stress signaling pathways and play important roles in response to these stresses.

## Comparative Expression Analysis of Rice and *Arabidopsis* AAT Genes

In order to explore valuable clues for the study of gene function, a comparative analysis of the expression patterns of rice and *Arabidopsis* AAT gene family was performed. With the exception of the expression data of *OsAAT* genes in pollens from MPSS tags, the other expression data of rice and *Arabidopsis* AAT genes were extracted from microarrays in root, leaf, inflorescence, pollen, seed/silique, and under abiotic stress. The ratios of the absolute values divided by the average of all microarray values were used for the analysis (Table S6).

Most of the rice and *Arabidopsis* AAT genes were found to be presented on at least one data set, while five genes (*OsCAT3*, *OsLAT6*, *AtANT4*, *AtVAAT10* and *AtVAAT5*) were absent from the two data sets (Figure 7). By integrating the data of microarray and MPSS tags, we found that 28 AAT genes were highly expressed in at least two organs examined and 21 AAT genes acted in tissue-specific manners. The expression levels of 36 AAT genes were extremely low in all examined organs. Six members of GAT subfamily and 4 of 6 in ProT subfamily were moderate and low expressed in all examined organs. On the contrary, nine members of AUX subfamily were high and/or moderate expressed in at least one organ except *OsAUX5* that were lowly expressed in all examined organs.

Meanwhile, this comparison also indicated that 26 AAT genes with close evolutionary relationships between rice and *Arabidopsis* showed similar expression patterns. Correlation coefficients between expression patterns ranged from 0.7 to 0.9 among most homologous genes (Table S7). For example, *AtLHT6*, *AtLHT3* and *OsLHT3* were very lowly expressed in all organs examined and evidently up-regulated in stems by drought and salt stresses (Figure 7B). *OsAUX2* and *AtLAX2* were mainly expressed in root, inflorescences and seeds and evidently down-regulated by salt stress (Figure 7E). *OsCAT1* and *AtCAT5* showed moderate or extremely low expression level except *OsCAT1* in leaf and were down-regulated by drought stress (Figure 7J). *OsANT2* and *AtANT2* were up-regulated by salt stress (Figure 7F), and OsBAT7 and AtBAT1 were down-regulated by cold stress (Figure 7K). More-
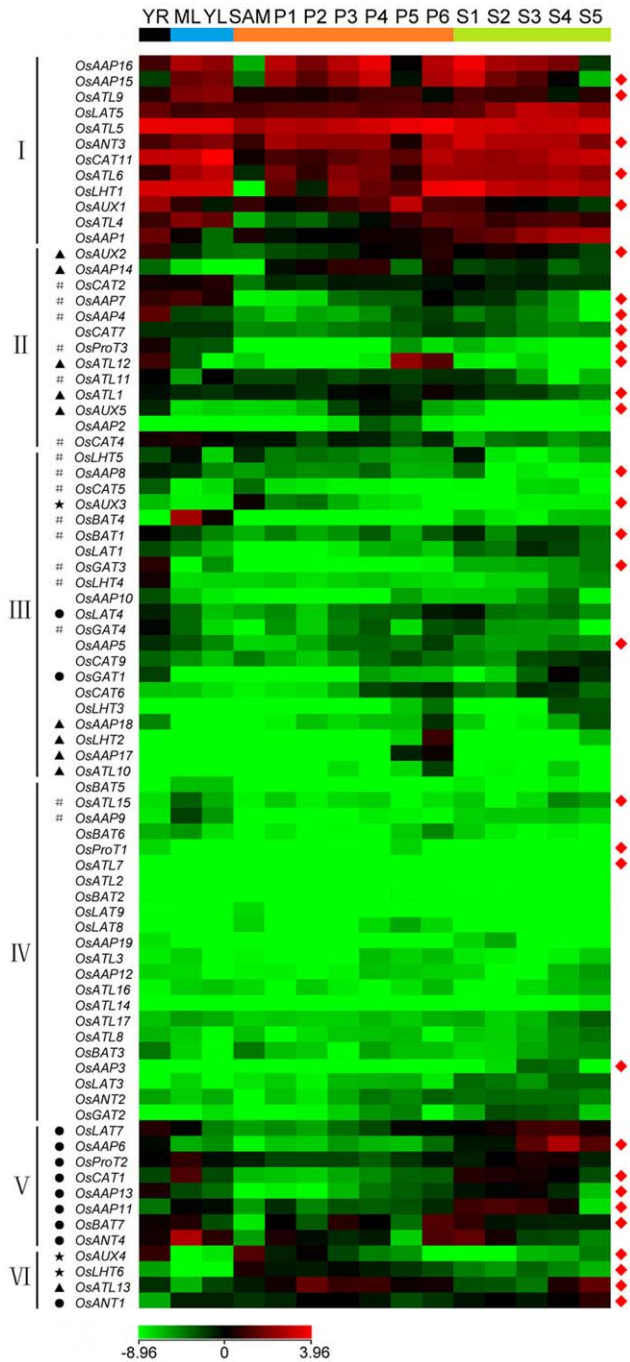
**Figure 4. Expression profiles of *OsAAT* genes in various organs.** The microarray data sets (GSE6893) of *OsAAT* genes expression in organs at various developmental stages were reanalyzed. A heat map representing hierarchical cluster in various organs are generated. Color key represents average log2 expression values of *OsAAT* genes. Samples are indicated at the top of each lane: YR, roots from 7-day-old seedlings; ML, mature leaf; YL, leaves from 7-day-old seedling; SAM, shoot apical meristem; different stages of panicle development: P1, 0-3 cm; P2, 3-5 cm; P3, 5-10 cm; P4, 10-15 cm; P5, 15-22 cm; P6, 22-30 cm; different stages of seed development: S1, 0-2 dap (day after pollination); S2, 3-4 dap; S3, 5-10 dap; S4, 11-20 dap; S5, 21-29 dap. Genes that share similar expression patterns are divided into six groups (?-?). Asterisks (*), hash symbols (#), triangles (▲) and rounds (●) indicate the genes with preferential expression level in SAM, YR and/or ML/YL, P1-P6 and S1-S5, respectively. The representative *OsAAT* genes differentially expressed in various organs for which real-time PCR analysis was performed are

indicated by a red diamond (?) on the right. The colour scale (representing average log signal values) is shown at the bottom. doi:10.1371/journal.pone.0049210.g004

over, *OsGAT1* and *AtGATL1* were moderately expressed in seeds (Figure 7C), *OsAUX1* and *AtAUX1* had high or moderate expression level in root, leaf, inflorescences and seeds (Figure 7D). *OsANT3* and *AtANT3* were highly expressed in all organs except *OsANT3* in pollens (Figure 7G), while OsATL1 and AtT5 were very lowly expressed in root, leaf and seeds (Figure 7H). *AtT3*, *OsATL4*, *OsATL5* and *OsATL6* were predominantly expressed in leaf, inflorescences and seeds (Figure 7I).

In addition, the expression of *OsAAT* genes in rice differed from those of their *Arabidopsis* homologs. For example, *OsLHT1* was highly expressed in root, leaf, inflorescences and seeds, while *AtLHT8* was only highly expressed in pollens, and had extremely low expression level in root, leaf, and seeds (Figure 7A). In conclusion, the expression patterns of genes provide foundation for future functional studies of *AAT* genes in both rice and *Arabidopsis*.

## Expression Pattern Divergence of Paralogous *OsAAT* Genes Involved in Duplication

The analysis on the expression pattern of *OsAAT* genes present in segment and in tandem duplication was performed. Out of the 12 pairs of segmentally duplicated genes, probe sets were available for 10 pairs on Affymetrix gene chip (http://signal.salk.edu/cgi-bin/RiceGE). The expression pattern was very much similar for 2 pairs of genes (*OsAAP7* and *OsAAP8*, *OsAUX1* and *OsAUX2*), indicating they were just formed due to duplication and retention of function (Figure 8A). The fate of 2 pairs (*OsATL3* and *OsATL4*, *OsCAT4* and *OsCAT11*) in Figure 8B could be described as nonfunctionalization, where one copy of the paralog had almost negligible expression in all organs, and which may be due to the fact that gene with low expression level would tend to lose its function in due course of evolution [47]. For the rest six pairs of paralogous genes (*OsAAP11* and *OsAAP16*, *OsGAT1* and *OsGAT2*, *OsANT1* and *OsANT2*, *OsANT3* and *OsANT4*, *OsATL5* and *OsATL6*, and *OsCAT1* and *OsCAT6*), the expression patterns were very divergent for one or more of the tissues detected, indicating neo-functionalization (Figure 8C).

Expression pattern analysis was also done for tandemly duplicated *OsAAT* genes. Two groups of genes, (*OsAAP2*, *OsAAP17* and *OsATL12*, *OsAAP15* and *OsAAP16*) had highly similar expression pattern and hence retention of expression (Figure 8D), whereas, for four groups of genes (*OsAAP4* and *OsAAP12*, *OsAAP5* and *OsAAP6*, *OsATL13* and *OsATL14*, *OsBAT2*, *OsBAT3*, *OsBAT4*, *OsBAT5* and *OsBAT6*), one copy of the paralog lost expression in all organs (Figure 8E). Furthermore, three pairs (*OsAAP3* and *OsAAP18*, *OsATL15* and *OsATL16*, *OsLAT3* and *OsLAT4*) showed divergent expression profiles (Figure 8F).

## Discussion

### Organization of *AAT* Family Genes in Rice

In previous reports, several *AAT* genes in *Arabidopsis* were classified and functionally characterized in detail, such as genes in the *AtAAP* subfamily [11,12,15], the *AtAUX* subfamily [33–37] and the *AtCAT* subfamily [39–42]. However, members of *AAT* family in rice are still unknown so far, and none of them is functionally characterized. In this study, we identified 85 *OsAAT* genes which were divided into nine subfamilies based on sequence similarity in amino acid, and there was obvious difference in numbers among subfamilies. The largest number found in *OsAAP* subfamily was nineteen, and the smallest found in *OsProT* subfamily was only
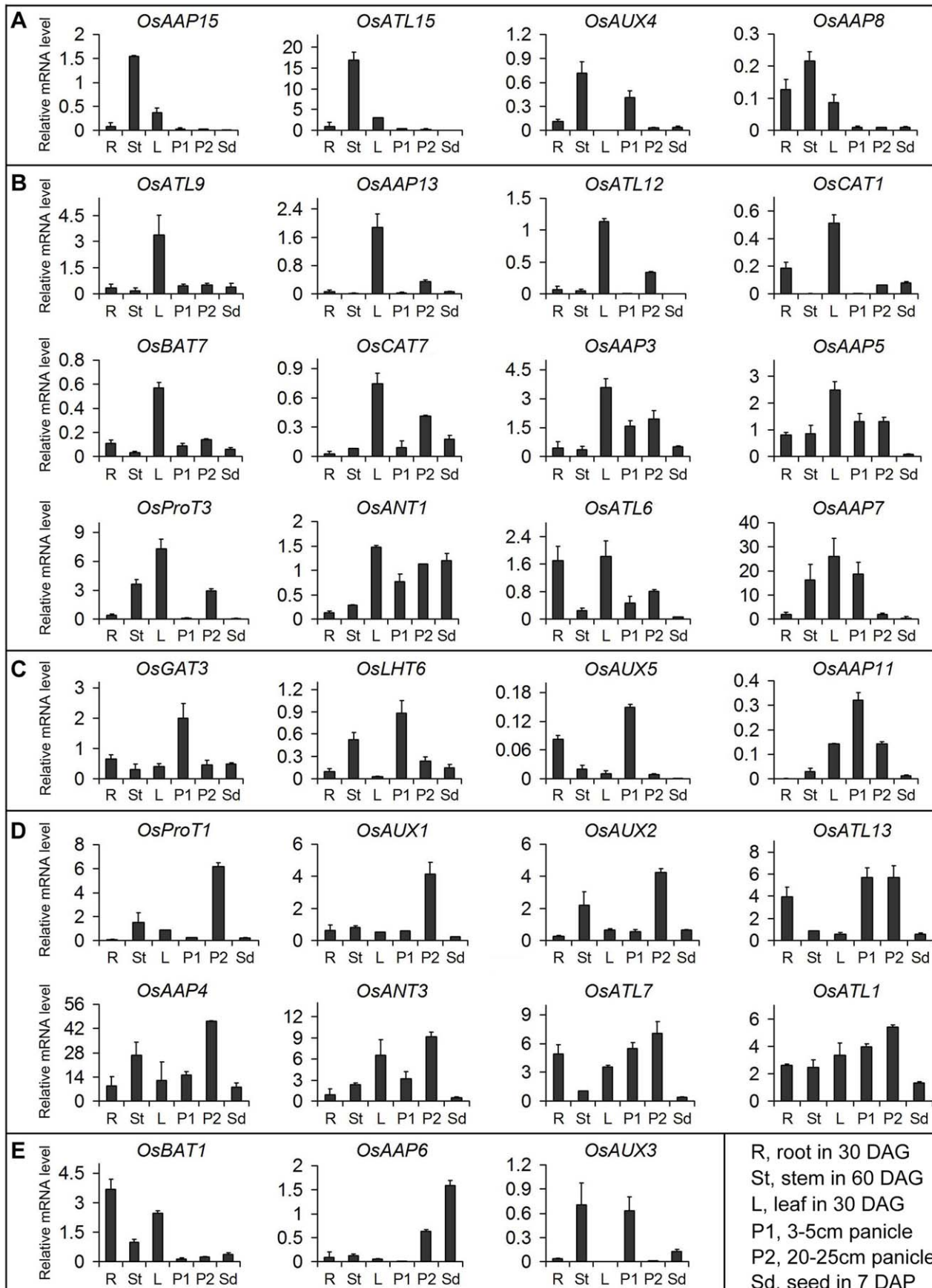
R, root in 30 DAG
St, stem in 60 DAG
L, leaf in 30 DAG
P1, 3-5cm panicle
P2, 20-25cm panicle
Sd, seed in 7 DAP

**Figure 5. Real-time PCR analysis of tissue-specific expression of the representative *OsAAT* genes.** Relative mRNA levels of individual genes normalized to *UBQ5* are shown. Y-axis shows the relative mRNA expression level; X-axis shows different organs. (A), (B), (C) and (D) Showing the genes with preferential expression in St, L, P1 and P2, respectively. (E) Showing the genes with preferential expression in R, Sd, St and P1. Error bars indicate standard deviations of independent biological replicates (n = 2 or more).
doi:10.1371/journal.pone.0049210.g005

three (Table 1). Combination phylogenetic analysis indicated that the rice *AAT* subfamilies were very good agreement with that in *Arabidopsis* (Figure S5), which revealed that *AAT* families in rice and *Arabidopsis* were formed before the split of monocotyledonous and dicotyledonous plants [4]. With the exception of *ATL*a and ATLb subfamilies, at least one gene in other nine subfamilies was functionally identified in *Arabidopsis*. In addition, due to the significant increase in the number of AAP subfamily genes and APC family genes, the number of 85 *OsAAT* members was far greater as compared to the number of 63 in *Arabidopsis* (Table S1). The increase in the number of *OsAAT* members reflects that expansion and rearrangement of the genome may be successfully undergone in the *OsAAT* family, and these transporters increased

in rice might play an important role in order to adapt to specific functions.

Chromosomal mapping of *OsAAT* family genes show their variable distribution on 12 rice chromosomes, but its most members are localized on chromosome 1, 4 and 12. Meanwhile, duplicated segments are mainly presented on chromosome 1, 3, 6 and 12, and nine gene groups in tandem duplication are localized only on five chromosomes (three, one, one, two and two groups on chromosome 1, 3, 4, 6 and 12, respectively) (Figure 1). Gene structure analysis reveals that most members in the same *OsAATs* subfamily are structurally conserved in the number of intron/exon and gene length (Figure S1), which indicates their close
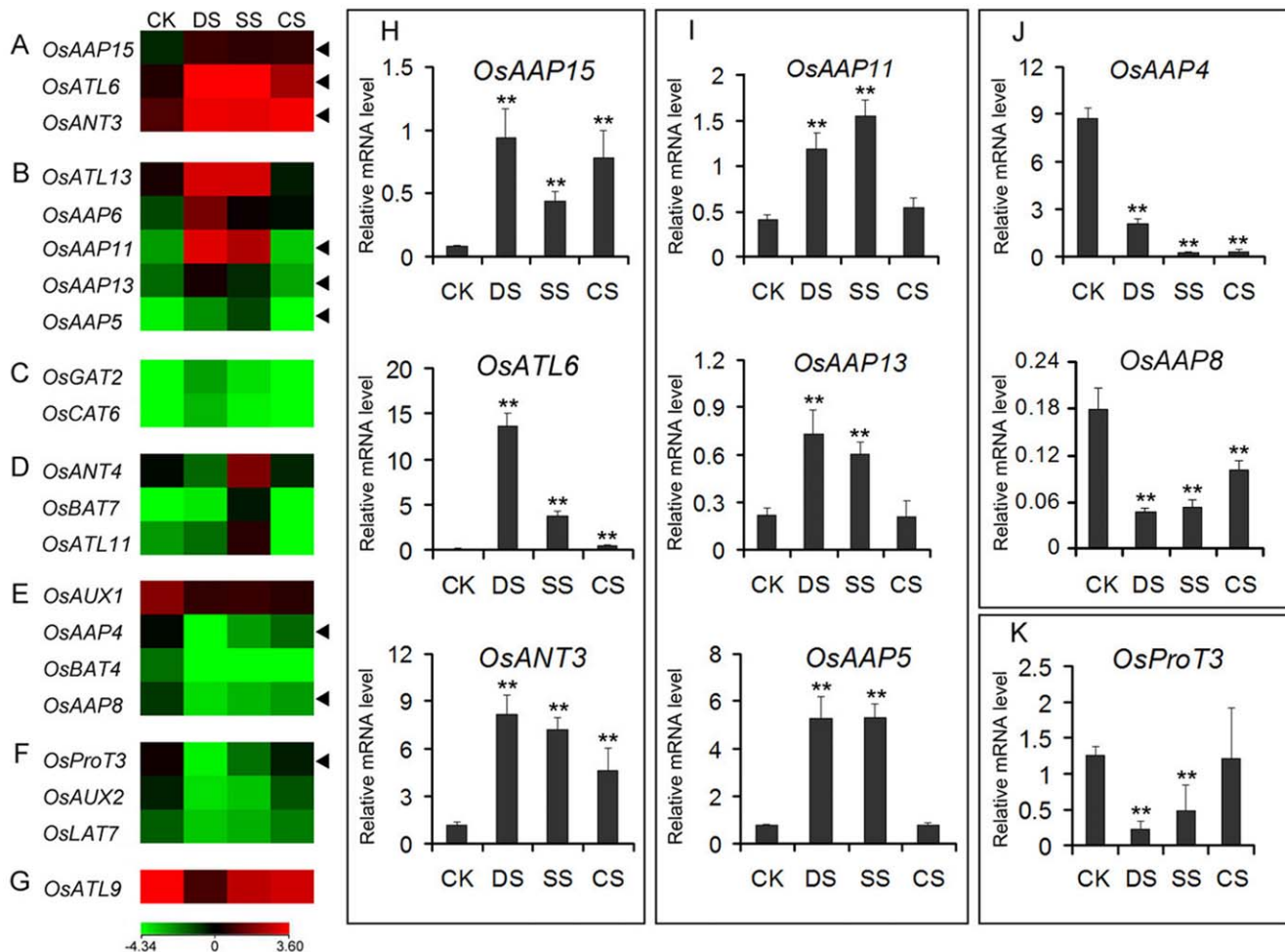


**Figure 6. Differential expression profiles of *OsAAT* genes under abiotic stresses.** The microarray data sets (GSE6901) of gene expression under various abiotic stresses were used for cluster display. The average log signal values of *OsAAT* genes under control and various stress conditions (indicated at the top of each lane) are presented by a heat map. Only those genes that exhibited >2-fold or more differential expression, under any of the given abiotic stress conditions, are shown. The color scale representing average log signal values is shown at the bottom. The representative *OsAAT* genes differentially expressed under different abiotic stresses for which real-time PCR analysis was performed are indicated by black triangle at the right. The mRNA levels for each candidate gene in different samples were calculated relative to its expression in control seedlings. Error bars indicate standard deviations of independent biological replicates (n = 3 or more). Two asterisks (**, $P < 0.01$, Student's *t*-test) represent significant differences between the controls and treatments. CK, control; DS, drought stress; SS, salt stress; CS, cold stress.
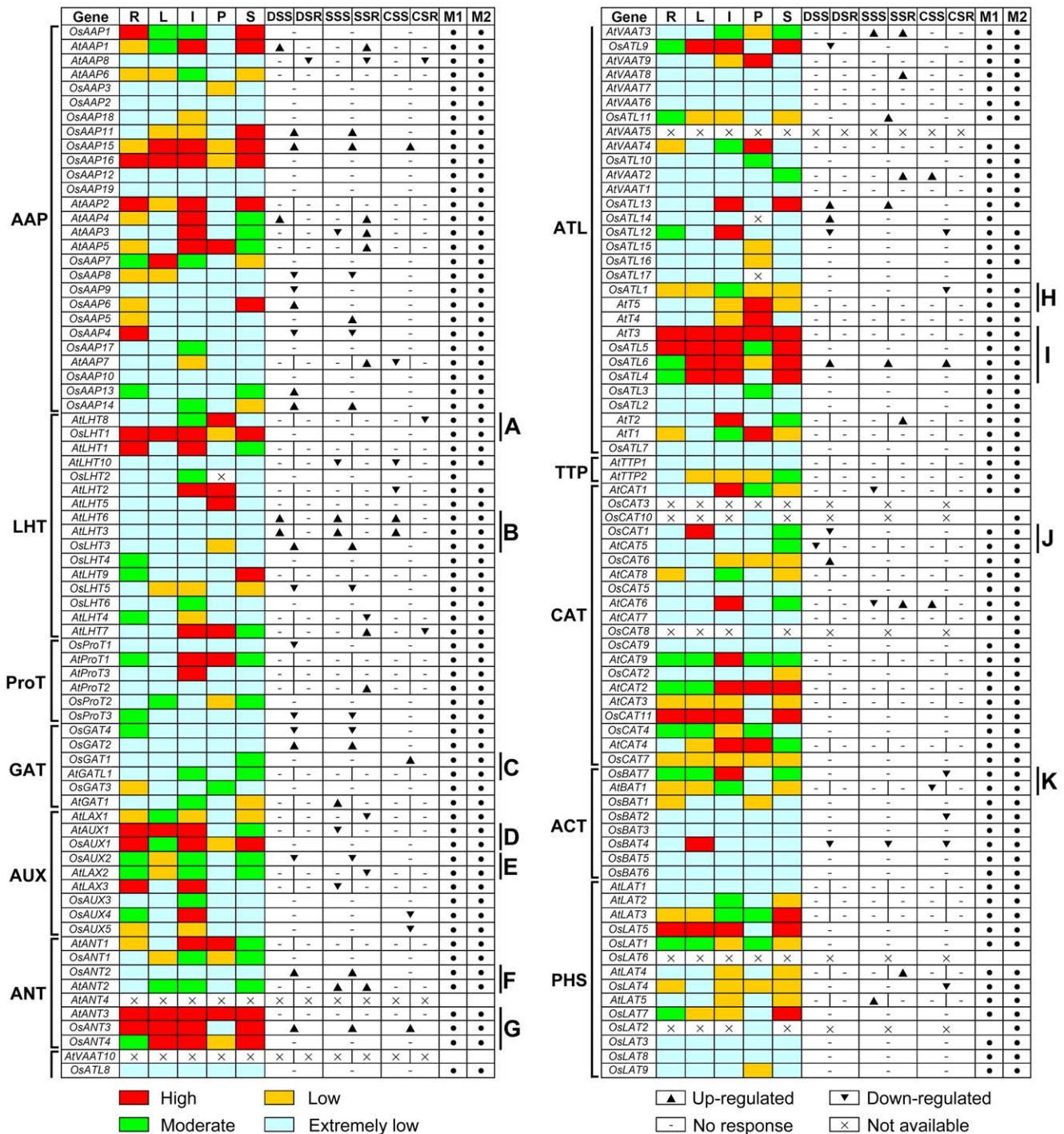doi:10.1371/journal.pone.0049210.g006

**Figure 7. Expression comparison between rice and *Arabidopsis AAT* genes in different organs and under abiotic stresses.** The *OsAAT* and *AtAAT* genes are displayed according to the order in the corresponding phylogenetic tree (Figure S5). The expression data of *OsAAT* genes in different organs are combined from microarrays (M1) and MPSS (M2). In microarrays and MPSS data, red, green, yellow and light blue boxes indicate high (more than 2, 300 tpm), moderate (between 1 and 2, between 50 and 300 tpm), low (between 0.5 and 1, the signature numbers between 0 and 50 tpm), and extremely low (less than 0.5, no signature) expression levels, respectively. The symbol "×" represents no probe or signature on microarray and MPSS. "▲", "▼" and "-" represent expression values that are evidently higher(>2), lower(<0.5) and no evident difference(0.5-2) under abiotic stresses compared to the control, respectively. (A) and (B-K) Showing homologous genes with distinct and similar expression patterns, respectively. R, root; L, leaf; I, inflorescence; P, pollen; S, silique (*Arabidopsis*) or seed (Rice); DSS and DSR; drought stressed shoot and root; SSS and SSR, salt stressed shoot and root; CSS and CSR, cold stressed shoot and root.
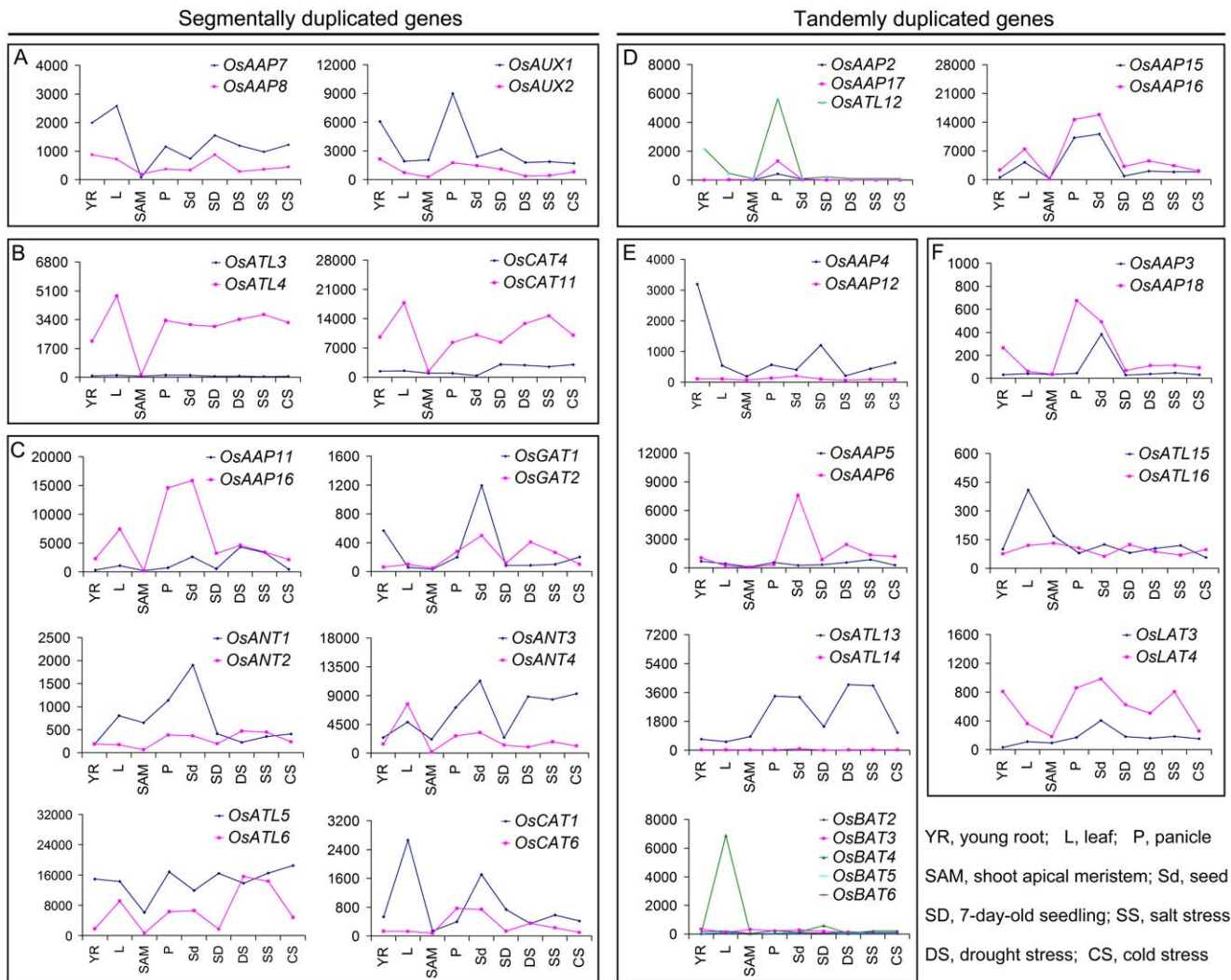doi:10.1371/journal.pone.0049210.g007

**Figure 8. Expression divergence of paralogous *OsAAT* genes involved in duplication.** The absolute values of duplicated genes obtained from microarray data were compared in various organs under abiotic stresses. X-axis indicates representative samples and Y-axis is scales of expression level. The segmentally and tandemly duplicated genes are shown on the left and the right, respectively. (A) and (D) Showing gene pairs that are retention of expression; (B) and (E) showing gene pairs described as non-functionalization; (C) and (F) showing gene pairs described as neo-functionalization.

doi:10.1371/journal.pone.0049210.g008

evolutionary relationship and the introduced classification of subfamilies.

## Duplication Contributed to the Expansion and Functional Divergence of *OsAATs* Family

Gene duplication is thought to be an important means of gene family expansion and functional diversity during evolution, which may occur through three major pathways: chromosomal segmental duplication, tandem duplication and retroposition [48–50]. In the present study, our analysis on gene duplication reveals that 47 of 85(55.30%) *OsAAT* genes are duplicated genes, 24 genes (28.24%) are involved in the segmental duplication, and 23 genes (27.06%) in tandem duplication, indicating that segmental and tandem duplications contribute almost equally to the expansion of the *OsAAT* gene family. Moreover, 16 genes in *OsAPC* family with 27 members are involved in duplication events, which may be largely responsible for the number difference of the member between *OsAPC* and *AtAPC* genes.

When gene duplication occurs, expression patterns and original functions of these genes may be retained [51]. Consistent with that, the comparison analysis for expression pattern of paralogous *OsAAT* genes involved in duplication indicates that *OsAAP7* and *OsAAP8*, and *OsAAP15* and *OsAAP16*, localized on segmental and tandem duplication, respectively, exhibited similar expression patterns in both development stages and abiotic stresses, indicating their overlapping functions (Figure 8A and 8D). However, it was well known that a great degree of expression and functional divergence might be present between two duplicated genes due to the intense selection pressure and the need for the diversification [49]. Most duplicated *OsAAT* genes exhibit distinct expression patterns and response to various abiotic stresses, such as *OsANT3* and *OsANT4*, *OsCAT1* and *OsCAT6* (segmentally duplicated genes) (Figure 8C); *OsAAP4* and *OsAAP12*, *OsLAT3* and *OsLAT4* (tandemly duplicated genes) (Figure 8E and 8F). These results suggested that chromosomal duplication events not only facilitated the expansion of the *OsAAT* family, and also lead to expression

divergences between duplicate genes, further contributing to the establishment of gene functional diversity during their evolution.

## Expression Patterns Divergence and Putative Function of OsAATs

The analysis on temporal and spatial expression patterns of OsAAT genes may provide useful information for establishing their putative functions [52–54]. Our microarray analysis showed that the expression patterns of the 80 OsAAT genes could be divided into six major groups. Some preferential or tissue-specific expression OsAAT genes were also identified. Only three (OsAUX3, OsAUX4 and OsLHT6) of the 80 genes were found to exhibit either preferential or tissue-specific expression in SAM, sixteen in YR and/or YL and/or ML. In addition, ten and eleven genes were preferentially or specifically expressed during different development stages of panicles and seeds, respectively. Interestingly, several genes were most highly expressed in several specific tissues, for example, OsATL12 were preferentially expressed in YR, P5 and P6, and OsCAT1 and OsANT4 had high expression level in ML, S1 and S2.

In Arabidopsis, AtAUX1 and AtLAX3 were primarily expressed in root and promoted lateral root formation [55,56]. Our data showed that OsAUX1, an ortholog of AtAUX1 and AtLAX3, had high expression levels in all the organs, especially in YR and P5, and OsAUX2, OsAUX4 and OsAUX5 were almost preferentially expressed in YR. Similar expression patterns suggest that these root preferentially or specifically expressed genes might play important roles in root formation and development. The results of some representative genes from real-time PCR showed that four genes (OsAAP8, OsAAP15, OsATL15 and OsAUX4) are predominantly or specifically expressed in stems. It is known that AtAAP2 may play critical role in the long-distance transport of amino acid [7,13]; AtAAP3 with mainly expression in root vascular tissue may be involved in amino acid uptake from phloem [57]; AtAAP6 might be responsible for amino acid uptake from xylem [58]. Therefore, combining their phylogenetic relationship and role of the AtAAPs, we infer that OsAAP8 and OsAAP15 might participate in the uptake and long-distance transport of amino acid.

Moreover, we also identified one gene (OsAAP6) that was preferentially expressed in early stages of seed development. AtAAP8 had been demonstrated that may be involved in amino acid uptake into the endosperm and supplying the developing embryo with amino acids during early embryogenesis [17]. It may be conjectured that these AAT members might also play critical roles in nutrient transport during seed development.

## Roles of OsAATs in Response to Abiotic Stresses and in Reproductive Development

The expression profile of a gene can provide a valuable clue for its functional study [53]. Our expression analysis by using qRT-PCR and/or microarray data reveal that a subset of OsAAT genes from different subfamilies shows significant and differential expression pattern under three abiotic stresses (Figure 6 and 7). It is known that amino acid transport is highly regulated by environmental signals, such as light, low temperature, high salt and/or drought [59]. The expression of AtAAP4 and AtAAP6 were reported to be down-regulated by salt stress in Arabidopsis [60], as well as McAAT2 in M. crystallinum [61]. On the contrary, AtProT2 [60], McAAT1 [61], and HvProT [62] were found to be strongly induced by salt stress. Similarly, in our investigation, seven genes (e.g. OsAAP4, OsAAP8, OsBAT4) and 11 genes (e.g. OsAAP6, OsAAP11, OsANT3) in OsAAT family were evidently down- and up-regulated, respectively, under salt and drought stresses (Figure 6).

The investigation suggests that the OsAAT genes may play a critical role in abiotic stress signaling in rice.

Many evidences demonstrate that there is an interaction between developmental processes and stress responses, and some genes may be co-regulated by both environmental factors and developmental cues [63]. It was reported that a network of rice genes are associated with stress response and seed development [64]. By expressional analysis from microarray data and qRT-PCR, we also found that several OsAAT genes (such as OsANT3, OsAAP6, OsAAP11, OsATL13 and OsAUX1), which were differentially expressed during at least one of the panicles and seed developmental stages, were significantly down- or up-regulated by one or more of the stress conditions. These genes may play important roles in plant growth and response to different abiotic stress conditions during reproductive development. This suggests that a number of OsAAT genes are likely to be involved in main developmental processes and stress responses. And their direct relationship requires further experimental validation.

## Conclusion

In conclusion, the results of this study display the genomic framework, classification, duplication manner and conserved motifs of the 85 OsAAT members, along with their expression profiles during different developmental stages as well as under abiotic stress treatments. These data will provide an insight into further understanding of functions of AAT members and their roles in rice growth and development. Our findings would be valuable in selecting candidate genes for functional validation studies of AAT members in rice. However, future research by adopting RNAi and overexpression strategies or insertion mutagenesis is required to explore the precise role of individual OsAAT gene.

## Materials and Methods

### Plant Materials and Abiotic Stress Treatment

To analyze the expression pattern of representative OsAAT genes, the rice seedlings of Nipponbare were grown under normal conditions in Wuhan University. The tissues and organs for expression pattern analysis were: (i) 30-day-old root (R, root) and leaf (L, leaf); (ii) 60-day-old stem(St); (iii) 3–5 cm panicles (P1); (iv) 20–25 cm panicles (P2); and (v) seed at 7 DAP (day after pollination) (Sd). For stress treatments, the 7-day-old seedlings were carefully transferred onto paper at 28°C as drought stress after which were air dried, placed in 400 mM NaCl solution at 28°C as salt stress, and kept in sterile water for 3h at 4°C as cold stress. Parallel control samples were prepared by keeping the seedlings in water for 3 h. All materials were collected and immediately frozen in liquid nitrogen, and stored in −80°C environment until RNA extraction.

### Database Screening and Identification of OsAATs

Several approaches were employed for the mining of all putative AAT members in the rice genome. Firstly, BLASTP searches of AAT domains (PF01490 and PF00324) search were performed on website of MSU-RGAP (http://rice. plantbiology. msu.edu/ domain_search shtml). Secondly, protein sequences of putative OsAAT members were downloaded from Search Interpro (http://www.ebi.ac.uk/interpro/ISearch?query = PF01490 and PF00324). Thirdly, key words "amino acid transporter" and "amino acid permease" were used as queries to search against Rice Genome Express Database (http://signal.salk.edu/cgi-bin/RiceGE). Resulting protein sequences were then used as queries to perform two database searches against MSU-RGAP (http://rice.

plantbiology.msu.edu/) and NCBI (http://www.ncbi.nlm.nih.gov/). After removing the redundant sequences, the remaining protein sequences were submitted to InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/) to confirm the existence of AAT domain. Information about full-length cDNA accessions, coding sequence length, gene structure for each gene and characteristics of proteins were obtained from KOME (Knowledge-based Oryza Molecular biological Encyclopedia) and MSU-RGAP. Gene structures of *OsAATs* were analyzed on the website of GSDS (Gene Structure Display Server) (http://gsds.cbi.pku.edu.cn/) [65]. To predict the putative TM regions in each OsAAT protein, the TMHMM Server v2.0 (http://www.cbs.dtu.dk/services/TMHMM/) was applied with default settings.

## Chromosomal Localization and Gene Duplication

*OsAAT* genes were mapped onto the corresponding rice chromosomes by identifying their chromosomal positions given in the RGAP (http://rice.plantbiology.msu.edu/analyses_search_locus.shtml). By using the MapChart software [66], the distribution of *OsAAT* genes on chromosomes was drawn and modified manually with annotation. *OsAAT* genes on duplicated chromosomal segments were explored by searching the segmental genome duplication of rice at RGAP segmental duplication database (http://rice.plantbiology.msu.edu/segmental_dup/500 kb/seg-dup_500 kb.shtml). Genes distributed nearby and separated by five or fewer genes were considered to be tandem duplicates.

## Phylogenetic Analysis and Sequence Alignment

The multiple sequence alignment was performed by using ClustalX version 1.83, and the unrooted phylogenetic tree based on the protein sequences of OsAAT members was constructed by neighbor-joining method. Bootstrap analysis was performed by using 1000 replicates. MEGA software version 4 [67] was used to display the phylogenetic tree. With the same method, the combined tree with OsAAT and AtAAT proteins was generated. The phylogeny of 85 OsAATs was also constructed by using the Bayesian estimation of MrBayes program [68]. The command "mcmc ngen = 1200000 samplefreq = 1200" were executed. When the calculation was finished, "the average standard deviation of split frequencies" was 0.0087. The program was stopped by typing "sump burnin = 250" and "sumt burnin = 250" and the phylogenetic tree was visualized through Treeview program.

The MEME motif search tool (http://meme.sdsc.edu/meme/intro.html) was used to identify motifs shared among related proteins within *OsAAT* gene family with default settings except that the maximum number of motifs was defined as 20 and the maximum width was set to 300. The sequence conservation of OsAAT subfamily members in amino acid was analyzed by DNAMAN software [52] and modified manually, and the conserved motifs and TM domains were annotated according to MEME analysis and TMHMM prediction, respectively.

## Expression Data Analysis of *OsAATs*

The EST, microarrays and MPSS expression data of *OsAAT* genes were extracted from UniGene database at NCBI (http://www.ncbi.nlm.nih.gov/unigene/), the Rice Functional Genomic Express Database (http://signal.salk.edu/cgi-bin/RiceGE) and the MPSS project (http://mpss.udel.edu), respectively. On the other hand, the expression data of *AtAATs* comparable to those used for rice were also downloaded (Table S3, S4 and S5). EST and MPSS data were used to detect expression pattern of *OsAATs* in different organs. The data of microarrays were used to analyze expression profiles of *OsAATs* in organs during different development stages (GSE6893) and under abiotic stresses (GSE6901). The absolute signal values were respectively divided by the average of all absolute values. The Cluster and Treeview software [68] were used to generate hierarchical cluster displays using the logarithmic values of the ratios in previous step. In expression comparative analysis of *OsAATs* and *AtAATs*, the genes that were up- or down-regulated at least two-fold with P<0.05 were considered to be differentially expressed.

## Quantitative Real-time PCR Analysis

To confirm the expression of representative *OsAAT* genes in rice organs, quantitative real-time PCR analysis was performed by SYBR-green fluorescence using gene-specific primers (Table S8). Primers were checked using dissociation curve analysis after the PCR reaction for their specificity. At least two or three independent biological replicates were made for real-time PCR analysis in different organs and under stress treatments, respectively. Three technical replicates were taken in each biological replicate. The first-strand cDNA was synthesized from DNaseI-treated total RNA using reverse transcriptase (ReverTra Ace, TOYOBO); 10-fold diluted cDNA samples were used for qRT-PCR. *TransStart* Eco Green qPCR SuperMix (TransGen, CHINA) was used to determine the expression levels for the genes in a Rotor-Gene Q real-time PCR machine (Qiagen). The following program was used: 9?°C for 30 s; 40 cycles of 95°C for 10 s, 55°C for 15 s, 72°C for 10 s. Rice *UBQ5* was used as an internal control gene [69]. The relative expression levels were analyzed by the standard curve method, three times diluted series of a mixed cDNA pools were selected to build a stand curve for each gene [68]. The given values of these diluted series are 1, 3, 9 and 27 (from low to high).

## Supporting Information

**Figure S1**  Structures of *OsAAT* genes. Gene structure analysis for 85 *OsAATs* is performed by using GSDS (http://gsds.cbi.pku.edu.cn/). The untranslated-regions (UTR), exons and introns are represented by gray boxes, black boxes and lines, respectively.
(TIF)

**Figure S2**  Prediction of the transmembrane regions of 85 OsAATs. The transmembrane regions of 85 OsAATs were predicted by using the TMHMM Server v2.0 (http://www.cbs.dtu.dk/services/TMHMM/) and displayed according to the order in Table 1.
(TIF)

**Figure S3**  **Chromosomal localization and duplication of *AtAAT* genes.** Chromosome numbers are indicated at the top of each bar. The scale on the left is in megabases (Mb). The cleavages on the chromosomes (vertical bars) indicate the position of centromeres.
(TIF)

**Figure S4**  **Bayesian phylogenetic analysis of OsAATs using MrBayes program.** Numbers at the nodes are posterior probability for MrBayes reconstructions. The numbers in AAP subfamily are marked by the letters.
(TIF)

**Figure S5**  **Phylogenetic analysis of OsAATs and AtAATs.** The phylogenetic tree of all AATs from *Arabidopsis* and rice after multiple sequence alignment using the full-length protein sequences is constructed by neighbor-joining method. The branches of different subfamilies are marked by different colors.
(TIF)

**Table S1  The general information and sequence characterization of 63 *AtAAT* genes.**
(DOC)

**Table S2  The MEME motif sequences and lengths in OsAAT proteins.**
(DOC)

**Table S3  The EST expression profiles of *OsAAT* genes.**
(DOC)

**Table S4  The microarray analysis of *OsAAT* genes in various organs under abiotic stresses.**
(DOC)

**Table S5  The MPSS analysis of *OsAAT* genes.**
(DOC)

**Table S6  Data for expression comparison of *OsAAT* and *AtAAT* genes.**
(DOC)

**Table S7  Correlation coefficients between expression patterns from homologous genes.**
(DOC)

**Table S8  Primers used in qRT-PCR of *OsAAT* genes.**
(DOC)

## Author Contributions

Conceived and designed the experiments: HMZ JZ. Performed the experiments: HMZ HLM LY XW. Analyzed the data: HMZ. Contributed reagents/materials/analysis tools: HMZ HLM LY XW. Wrote the paper: HMZ JZ.

## References

1. Tegeder M, Offler CE, Frommer WB, Patrick JW (2000) Amino acid transporters are localized to transfer cells of developing pea seeds. Plant Physiol 122: 319–326.
2. Wipf D, Ludewig U, Tegeder M, Rentsch D, Koch W, et al. (2002) Conservation of amino acid transporters in fungi, plants and animals. Trends Biochem Sci 27: 139–147.
3. Tegeder M (2012) Transporters for amino acids in plant cells: some functions and many unknowns. Curr Opin Plant Biol 15: 1–7.
4. Rentsch D, Schmidt S, Tegeder M (2007) Transporters for uptake and allocation of organic nitrogen compounds in plants. FEBS Lett 581: 2281–2289.
5. Saier MH, Yen MR, Noto K, Tamang DG, Elkan C (2009) The Transporter Classification Database: recent advances. Nucleic Acids Res 37: D274–D278.
6. Ortiz-Lopez A, Chang HC, Bush DR (2000) Amino acid transporters in plants. Biochim Biophys Acta 1465: 275–280.
7. Okumoto S, Pilot G (2011) Amino acid export in plants: a missing link in nitrogen cycling. Mol Plant 4: 453–463.
8. Fischer WN, Andre B, Rentsch D, Krolkiewicz S, Tegeder M, et al. (1998) Amino acid transport in plants. Trends Plant Sci 3: 188–195.
9. Hunt E, Gattolin S, Newbury HJ, Bale JS, Tseng HM, et al. (2010) A mutation in amino acid permease AAP6 reduces the amino acid content of the *Arabidopsis* sieve elements but leaves aphid herbivores unaffected. J Exp Bot 61: 55–64.
10. Fischer WN, Kwart M, Hummel S, Frommer WB (1995) Substrate specificity and expression profile of amino acid transporters (AAPs) in *Arabidopsis*. J Biol Chem 270: 16315–16320.
11. Okumoto S, Schmidt R, Tegeder M, Fischer WN, Rentsch D, et al. (2002) High affinity amino acid transporters specifically expressed in xylem parenchyma and developing seeds of *Arabidopsis*. J Biol Chem 277: 45338–45346.
12. Couturier J, Faÿ ED, Fitz M, Wipf D, Blaudez D, et al. (2010) PtAAP11, a high affinity amino acid transporter specifically expressed in differentiating xylem cells of poplar. J Exp Bot 61: 1671–1682.
13. Hirner B, Fischer WN, Rentsch D, Kwart M, Frommer WB (1998) Developmental control of H+/amino acid permease gene expression during seed development of *Arabidopsis*. Plant J 14: 535–544.
14. Lee YH, Foster J, Chen J, Voll LM, Weber APM, et al. (2007) AAP1 transports uncharged amino acids into roots of *Arabidopsis*. Plant J 50: 305–319.
15. Sanders A, Collier R, Trethewy A, Gould G, Sieker R, et al. (2009) AAP1 regulates import of amino acids into developing *Arabidopsis* embryos. Plant J 59: 540–552.
16. Svennerstam H, Ganeteg U, Näsholm T (2008) Root uptake of cationic amino acids by *Arabidopsis* depends on functional expression of amino acid permease 5. New Phytol 180: 620–630.
17. Schmidt R, Stransky H, Koch W (2007) The amino acid permease AAP8 is important for early seed development in *Arabidopsis thaliana*. Planta 226: 805–813.
18. Koch W, Kwart M, Laubner M, Heineke D, Stransky H, et al. (2003) Reduced amino acid content in transgenic potato tubers due to antisense inhibition of the leaf H+/amino acid symporter StAAP1. Plant J 33: 211–220.
19. Miranda M, Borisjuk L, Tewes A, Heim U, Sauer N, et al. (2001) Amino acid permeases in developing seeds of *Vicia faba* L.: expression precedes storage protein synthesis and is regulated by amino acid supply. Plant J 28: 61–71.
20. Tan Q, Grennan AK, Pélissier HC, Rentsch D, Tegeder M (2008) Characterization and expression of French bean amino acid transporter PvAAP1. Plant Sci 174: 348–356.
21. Chen LS, Bush DR (1997) LHT1, lysine- and histidine-specific amino acid transporter in *Arabidopsis*. Plant Physiol 115: 1127–1134.
22. Hirner A, Ladwig F, Stransky H, Okumoto S, Keinath M, et al. (2006) *Arabidopsis* LHT1 is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. Plant Cell 18: 1931–1946.
23. Svennerstam H, Ganeteg U, Bellini C, Näsholm T (2007) Comprehensive screening of *Arabidopsis* mutants suggests the lysine histidine transporter 1 to be involved in plant uptake of amino acids. Plant Physiol 143: 1853–1860.
24. Svennerstam H, Jämtgård S, Ahmad I, Huss-Danell K, Näsholm T, et al. (2011) Transporters in *Arabidopsis* roots mediating uptake of amino acids at naturally occurring concentrations. New Phytol 183: 1–9.
25. Grallath S, Weimar T, Meyer A, Gumy C, Suter-Grotemeyer M, et al. (2005) The AtProT family. compatible solute transporters with similar substrate specificity but differential expression patterns. Plant Physiol 137: 117–126.
26. Lehmann S, Funck D, Szabados L, Rentsch D (2010) Proline metabolism and transport in plant development. Amino Acids 39: 949–962.
27. Lehmann S, Gumy C, Blatter E, Boeffel S, Fricke W, et al. (2011) In planta function of compatible solute transporters of the AtProT family. J Exp Bot 62: 787–796.
28. Schwacke R, Grallath S, Breitkreuz KE, Stransky E, Stransky H, et al. (1999) LeProT1, a transporter for proline, glycine betaine, and gamma-amino butyric acid in tomato pollen. Plant Cell 11: 377–392.
29. Igarashi Y, Yoshiba Y, Takeshita T, Nomura S, Otomo J, et al. (2000) Molecular cloning and characterization of a cDNA encoding praline transporter in rice. Plant Cell Physiol 41: 750–756.
30. Ueda A, Shi W, Shimada T, Miyake H, Takabe T (2008) Altered expression of barely praline transporter causes different growth response in *Arabidopsis*. Planta 227: 277–286.
31. Meyer A, Eskandari S, Grallath S, Rentsch D (2006) AtGAT1, a high affinity transporter for gamma-aminobutyric acid in *Arabidopsis thaliana*. J Biol Chem 281: 7197–7204.
32. Chen LS, Ortiz-Lopez A, Jung A, Bush DR (2001) ANT1, an aromatic and neutral amino acid transporter in *Arabidopsis*. Plant Physiol 125: 1813–1820.
33. Bennett MJ, Marchant A, Green HG, Ward SP, Millner PA, et al. (1996) *Arabidopsis* AUX1 gene: a permease-like regulator of root gravitropism. Science 273: 948–950.
34. Marchant A, Kargul J, May ST, Muller P, Delbarre A, et al. (1999) AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root apical tissues. EMBO J 18: 2066–2073.
35. Marchant A, Bhalerao R, Casimiro I, Eklöf J, Casero PJ, et al. (2002) AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the *Arabidopsis* seedling. Plant Cell 14: 589–597.
36. Swarup R, Kargul J, Marchant A, Zadik D, Rahman A, et al. (2004) Structure-function analysis of the presumptive *Arabidopsis* auxin permease AUX1. Plant Cell. 16, 3069–3083.
37. Swarup K, Benková E, Swarup R, Casimiro I, Péret B, et al. (2008) The auxin influx carrier LAX3 promotes lateral root emergence. Nat Cell Biol 10: 946–954.
38. Hoyerová K, Perry L, Hand P, Laňková M, Kocábek T, et al. (2008) Functional characterization of PaLAX1, a putative auxin permease, in heterologous plant systems. Plant Physiol 146: 1128–1141.
39. Frommer WB, Hummel S, Unseld M, Ninnemann O (1995) Seed and vascular expression of a high-affinity transporter for cationic amino acids in *Arabidopsis*. Proc Natl Acad Sci USA 92: 12036–12040.
40. Su YH, Frommer WB, Ludewig U (2004) Molecular and functional characterization of a family of amino acid transporters from *Arabidopsis*. Plant Physiol 136: 3104–3113.
41. Dündar E, Bush DR (2009) BAT1, a bidirectional amino acid transporter in *Arabidopsis*. Planta 229: 1047–1056.
42. Michaeli S, Fait A, Lagor K, Nunes-Nesi A, Grillich N, et al. (2011) A mitochondrial GABA permease connects the GABA shunt and the TCA cycle, and is essential for normal carbon metabolism. Plant J 67: 485–498.
43. Shen CJ, Bai YH, Wang SK, Zhang SN, Wu YR, et al. (2010) Expression profile of PIN, AUX? LAX and PGP auxin transporter gene families in Sorghum bicolor under phytohormone and abiotic stress. FEBS Lett 277: 2954–2969.
44. Qi YH, Wang SK, Shen CJ, Zhang SN, Chen Y, et al. (2011) OsARF12, a transcription activator on auxin response gene, regulates root elongation and affects iron accumulation in rice (*Oryza sativa* L.). New Phytol 193: 109–120.

45. Komatsu K, Maekawa M, Ujiie S, Satake Y, Furutani I, et al. (2003) LAX and SPA: Major regulators of shoot branching in rice. Proc Natl Acad Sci USA 100: 11765–11770.

46. Tabuchi H, Zhang Y, Hattori S, Omae M, Shimizu-Sato S, et al. (2011) LAX PANICLE2 of rice encodes a novel nuclear protein and regulates the formation of axillary meristems. Plant Cell 23: 3276–3287.

47. Tegeder M, Ward JM (2012) Molecular evolution of plant AAP and LHT amino acid transporters. Front Plant Sci 3: 21.

48. Lynch M, Conery JS (2000) The evolutionary fate and consequences of duplicated genes. Science 290: 1151–1155.

49. Prince VE, Pickett FB (2002) Splitting pairs: the diverging fates of duplicated genes. Nat Rev Genet 3: 827–837.

50. He X, Zhang J (2005) Rapid sub-functionalization accompanied by prolonged and substantial neo-functionalization in duplicate gene evolution. Genetics 169: 1157–1164.

51. Kong H, Landherr LL, Frohlich MW, Leebens-Mack J, Ma H, et al. (2007) Patterns of gene duplication in the plant SKP1 gene family in angiosperms: evidence for mutiple mechanisms of rapid gene birth. Plant J 50: 873–885.

52. Ma H, Zhao J (2010) Genome-wide identification, classification, and expression analysis of the arabinogalactan protein gene family in rice (Oryza sativa L.). J Exp Bot 61: 2647–2668.

53. Zhao X, Huang J, Yu H, Wang L, Xie W (2010) Genomic survey, characterization and expression profile analysis of the peptide transporter family in rice (Oryza sativa L.). BMC Plant Biol 10: 92–111.

54. Singh A, Baranwal V, Shankar A, Kanwar P, Ranjan R, et al. (2012) Rice phospholipase A superfamliy: organization, phylogenetic and expression analysis during abiotic stresses and development. PLoS ONE 7: e30947.

55. Yang YD, Hammes UZ, Taylor CG, Schachtman DP, Nielsen E (2006) High-affinity auxin transport by the AUX1 influx carrier protein. Curr Biol 16: 1123–1127.

56. Ugartechea-Chirino Y, Swarup R, Swarup K, Péret B, Whitworth M, et al. (2010) The AUX1 LAX family of auxin influx carriers is required for the establishment of embryonic root cell organization in Arabidopsis thaliana. Ann Bot 105: 277–289.

57. Okumoto S, Koch W, Tegeder M, Fischer WN, Biehl A, et al. (2004). Root phloem-specific expression of the plasma membrane amino acid proton co-transporter AAP3. J Exp Bot 55: 2155–2168.

58. Fischer WN, Loo DD, Koch W, Ludewig U, Boorer KJ, et al. (2002) Low and high affinity amino acid H$^+$-cotransporters for cellular import of neutral and charged amino acids. Plant J 29: 717–731.

59. Liu X, Bush DR (2006) Expression and transcriptional regulation of amino acid transporters in plants. Amino Acids 30: 113–120.

60. Rentsch D, Hirner B, Schmelzer E, Frommer WB (1996) Salt stress induced proline transporters and salt stress-repressed broad specificity amino acid permeases identified by suppression of a yeast amino acid permease-targeting mutant. Plant Cell 8: 1437–1446.

61. Popova OV, Dietz KJ, Golldack D (2003) Salt-dependent expression of a nitrate transporter and two amino acid transporter genes in Mesembryanthemum crytallinum. Plant Mol Biol 52: 569–578.

62. Ueda A, Shi W, Sanmiya K, Shono M, Takabe T (2001) Functional analysis of salt-induced proline transporter of barely roots. Plant cell Physiol 42: 1282–1289.

63. Jain M, Nijhawan A, Arora R, Agarwal P, Ray S, et al. (2007) F-box proteins in rice: Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. Plant Physiol 143: 1467–1483.

64. Cooper B, Clarke JD, Budworth P, Kreps J, Hutchison D, et al. (2003) A network of rice genes associated with stress response and seed development. Proc Natl Acad Sci USA 100: 4945–4950.

65. Liu F, Chang XJ, Ye Y, Xie WB, Wu P, et al. (2011). Comprehensive sequence and whole-life-cycle expression profile analysis of the phosphate transporter gene family in rice. Mol Plant 4: 1105–1122.

66. Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J hered 93: 77–78.

67. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596–1599.

68. Ma H, Zhao H, Liu Z, Zhao J (2011) The Phytocyanin gene family in rice (Oryza sativa L.): genome-wide identification, classification and transcriptional analysis. PLoS ONE 6: e25184.

69. Jain M, Nijhawan A, Tyagi AK, Khurana JP (2006) Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. Biochem Biophys Res Commun 345: 646–651.